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Stochastic Life-History Variation in Populations of Western Ribbon Snakes (Thamnophis Proximus) in East Texas

Jeremy Chamberlain

STOCHASTIC LIFE-HISTORY VARIATION IN POPULATIONS OF WESTERN RIBBON SNAKES (*THAMNOPHIS PROXIMUS)* IN EAST TEXAS

By

JEREMY CHAMBERLAIN

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Neil B. Ford, Ph.D., Committee Chair

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This is to certify that the Master's Thesis of

JEREMY CHAMBERLAIN

has been approved for the thesis requirement on
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for the Master of Science degree

Approvals:

Thesis Chair: Neil B. Ford, Ph.D.

Member: Srini Kambhampati, Ph.D.

Member: Ali Azghani, Ph.D.

Chair, Department of Biology

Dean, College of Arts and Sciences

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Abstract

STOCHASTIC LIFE-HISTORY VARIATION IN POPULATIONS OF WESTERN RIBBON SNAKES (*THAMNOPHIS PROXIMUS)* IN EAST TEXAS

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Understanding variation in life-history strategies can be important when determining the influence of an organism's environment on its natural history. Variation in strategies can play a significant role on the overall fitness and survival of an individual. Some environmental conditions may influence this variation more than others. In particular, stochastic environments, such as a floodplain, may have large impacts on life-history strategies of organisms living within them. Floodplains are known to have high rates of extrinsic mortality. Theoretically this should shift life-history traits towards a "fast-type" strategy, with fast growth rates, high annual fecundity, and larger mean body-sizes. To test this hypothesis, this study examined life-history strategies, mainly age-structure, body-size, and reproduction of the western ribbon snake (*Thamnophis proximus*) in East Texas. Two sites within East Texas were examined to determine the influence of floodplains on life-history traits. One was a bottomland forest within the floodplain of the Sabine River, Smith County, Texas. The other was stable lake/pond

ecosystem also within Smith County, Texas. We were unable to detect any differences in age-structure or body-size between either population of ribbon snakes in East Texas. Additionally, the reproductive characteristics (mean offspring SVL, mean offspring mass, clutch size, and clutch mass) we measured did not correlate with number of floods, indicating little influence of stochasticity on these traits. The lack of variation in these life-history traits may be attributed to small sample sizes and the use of skeletochronology to assess age-structure. Skeletochronology has not been validated as a tool for measuring age-structure in East Texas. This study also investigates the efficacy of skeletochronology in East Texas using four novel validation techniques.

Chapter One

Overview of life-history theory

Life-histories are defined as, "the significant features of the life-cycle through which an organism passes, with particular reference to strategies influencing survival and reproduction" (Lincoln and Boxshall, 1987). Suites of in life-history traits often, sometimes referred to as life-history strategies, work together to help an organism survive and reproduce in its specific environment. Characteristics such as growth rate, age/size to first reproduction, number of young, reproductive mass, yearly and total fecundity, and many other traits (Stearns, 1976; Stearns, 1977). These characteristics are vitally important to individuals, as they "decide" when, how, and how much of their genetic material will be passed to following generations. Variation in any one of these characteristics can have a significant influence on the overall fitness and survival of an individual, influencing the demography of populations. Most of these characteristics are correlated with one another, and often act in parallel or antagonistically. A change in one characteristic can markedly influence, positively or negatively, one or more other life-history traits

Correlated relationships between life-history characteristics are hypothesized because they relate to allocation of fixed energy resources of an organism, for which these characteristics depend (Bronikowski & Arnold, 1999; Lochmiller & Deerenberg, 2000; Luiselli et al., 1996; Partridge & Harvey, 1988; Preziosi et al., 1996; Reznick et al.,

2002; Reznick et al., 2000; Stearns, 1989). In other words, to increase one characteristic, e.g. clutch-size, an organism must allocate more of its total energy to producing more individual offspring. But, because there is often only a fixed amount of energy resources over the lifetime of an organism to invest more resources to clutch-size requires the removal of energy from another trait, for example offspring size. This means that even though there are more individual offspring, each of them will have smaller body mass. Of course, significantly changing the allocation of these resources can dramatically affect the fitness of the inidividual. In a cold environment, for example, having many small offspring may increase the chance of each of the offspring dying due to heat loss because the offspring would be smaller. Increasing the weight of each offspring would help the offspring retain heat to survive, but consequently decrease the clutch size (Bernardo, 1996; Berven & Gill, 1983; Blouin-Demers & Weatherhead, 2007; Brown & Shine, 2007; Ford & Seigel, 1989b). The basis of life-history theory revolves around the idea that individuals should produce whatever combination results in the highest fitness over their lifetime.

Traditionally, life-history traits were quantified in terms of age and body size. Both of these measurements have unique implications on a given life-history trait (Castanet & Baez, 1988; Davidowitz et al., 2005; Partridge & Harvey, 1988; Preziosi et al., 1996; Wilbur & Rudolf, 2006). For instance, when asking the question "When do individuals in this population begin reproducing?" one might measure the youngest age at which individuals are sexually mature or measure the smallest length and weight of sexually mature individuals within a population. Whereas length and weight may give an "absolute" measure of when individuals are sexually mature, these data cannot convey

how long it took individuals to reach that size, which in some cases may be the more relevant question. This is particularly true in environments where yearly mortality is high and an individual may not survive to reproduce the following year. This makes it adaptive to reach a reproducing size faster, i.e., increasing growth rate (Bronikowski & Arnold, 1999; Stearns, 1976). The idea of growth rate, in particular, is important to understand because it controls when many other life-history traits will occur, such as reproduction (Caley & Schwarzkopf, 2004; Stearns, 1976, 1977).

Growth rate is a primary determinant of age at first and fecundity. Large body-size resulting from a faster growth rate is correlated with increased fecundity or larger offspring size (Bronikowski, 2000; Ford & Seigel, 1989b) . In some species a critical body-size must be reached before reproduction can occur (Byars et al., 2010). Understanding not only what that critical body-size is but also how long it takes to get to that size is important for life-histories. Timing of first reproduction isn't simply a function of how large an individual is but when it reaches sexual maturity. Some individuals might need to wait an entire extra year to reproduce because they were not large enough, during which they may overshoot that critical size. Ideally in any study, both age and body size would be measured to address questions of life-history evolution, such as sexual maturity.

In different environments natural selection often favors particular suites of life-history traits that are suited for each these habitats (Berven, 1982; Bronikowski & Vleck, 2010; Bronikowski & Arnold, 1999; Carriere & Roff, 1995; Day et al., 2002; Harvey & Cluttonbrock, 1985; Stearns, 1976; Wilbur & Rudolf, 2006). Organisms cannot adapt their life-history strategies unless there is variation available to select. Clearly, having

variation in life-history characteristics and strategies can be adaptive for individuals in certain environments (Shine & Brown, 2008). Most all traits have some variation between the major taxa of organisms. However, most traits have variation even at the population level within a given species. Some groups have more variation than others, and may be linked with the stability of environments or may be due to the amount of genetic material available for selection. This variation may be pushed by many environmental factors but can be explained at the organismal level through two main functions. Individuals, populations, species, etc. can vary genetically and therefore express variation in life-history traits. These groups can differentially regulate and express the same genes to various degrees also resulting in variation in life-history traits. This is considered phenotypic plasticity (Ford & Seigel, 1989a; Nylin & Gotthard, 1998; Roff & Gelinas, 2003; Scheiner, 1993; Seigel & Ford, 1991; Stearns & Koella, 1986).

Those environmental factors that influence the variation in life-history traits include: food, predation, climate, competition, habitat availability, and environmental stochasticity. Many of these factors are inter-related. For instance, a change in climate such as rainfall can alter the amount of food, habitat availability, and stochasticity. Changes in food/nutrient availability alter an organism's ability to gain and store energy that it can use towards reproduction, growth, or survival (Martin, 1987; Seigel & Ford, 1992). Increased predation lowers the probability that individuals in a population will survive to the following year (or next opportunity to mate) and therefore puts pressure to have reproductive events occur sooner (Magnhagen, 1991; Reznick & Endler, 1982; Spitze, 1991). Air temperatures, precipitation, humidity, wind, seasonality, and many other interacting factors produce climate. These in turn can be the deciding factors for

many life-history traits such as circannual rhythms, circadian rhythms, and timing of other important life stages, such as metamorphosis (Chaloupka, 2001; Franklin et al., 2000) . Climate also influences other factors like food availability and habitat availability. Inter- and intra-specific competition limits the amount of resources to which an individual has access and therefore may limit the amount of energy it can dedicate to reproduction (Herms & Mattson, 1992; Huston & Smith, 1987; Stearns, 1977). Furthermore, having to "fight" to protect resources from other competitors also requires energy. The consequences of this kind of competition pressure may be difficult to predict, however, because there are many kinds of competition. Similarly, the consequences of limited habitat availability on life-history traits can be difficult to predict (Morey & Reznick, 2004; Perfito et al., 2007).

Influence of stochasticity of life-history evolution

One factor that underlies these environmental pressures that shape life-history is stochasticity, or unpredictability. Temperature, food availability, flooding, drought, fire, and habitat availability often vary periodically but unpredictably. For instance, flooding in certain habitats may be a yearly phenomenon, but the number of floods, the extent of those floods, and resulting changes to the landscape a Stochasticity alone can cause variation in life-history traits sometimes in a predictable manner (Perfito et al. 2007; (Bardsen et al., 2011; Bradford & Roff, 1993; Bronikowski & Vleck, 2010; Chan & Zamudio, 2009; Childs et al., 2004; Hampton & Ford, 2007; Lande, 1993; Saether, 1997; Stearns, 1976; Stearns, 1989; Wilbur & Rudolf, 2006). Fires on a prairie, for instance, do not happen in a predictable fashion but happen often enough that many plants growing in those prairies depend on fires for reproduction and germination (Huston & Smith, 1987;

Kirkman et al., 1998; Menges & Dolan, 1998; Overbeck & Pfadenhauer, 2007). Phenotypic variation, whether genetically or environmentally based, is crucial for populations living in environments that are highly stochastic. Strict conformity to one life-history strategy may result in the loss of the majority of a population during one stochastic event (Penteriani et al., 2005). If for instance, a population could only breed at one specific time in a year, and some large-scale stochastic event such as drought prevented any individual from reproducing, the consequences could be drastic. The wood frog (*Rana sylvatica*) is a good example of this. They are mass breeders that perform much of their reproduction during a relatively short amount of time (often less than 2 or 3 weeks) (Morrison & Hero, 2003). Rittenhouse et al. (2009) showed that drought and timber harvest markedly reduced reproduction and increased mortality in a northern population of these frogs. Thus varying the time of reproduction allows enough individuals to survive and reproduce the next generation.

Generally, stochastic environments tend to cause high mortality in a population. As a result, those individuals should have predictably faster growth and reproduction with a greater effort exhibited each reproductive attempt compared to those in relatively more stable environments (Stearns, 1976; Stearn, 1977). Essentially, the idea is that an individual should accomplish as much reproduction as quickly as it can because the chance of surviving to reproduce again is low. The trade-off of course is that little energy is stored for future reproductive events.

Age and body-size

In order to analyze the variation due to environmental stochasticity in traits and its implications for individual fitness, quantifiable measures must be used. Age and body

size of individuals are two standard characteristics that many researchers have used to quantify life-history traits. Often it is difficult to estimate the age of organisms (Halliday & Verrell, 1988; Kirkpatrick, 1984; Kozlowski, 1992; Kozlowski & Weiner, 1997; Shine et al., 1998). However, three standard methods are often used: mark-recapture studies, size-frequency data, and skeletochronology (Halliday & Verrell, 1988; McNamara & Houston, 1996). With the help of technologies, other methods are currently being developed, including rate of telomere decay in DNA (Kruk et al., 1995). The traditional methods of aging are all labor-intensive and some require longer-term trials. As the name suggests, mark-recapture studies require the release of already known aged individuals, typically neonates, over several years and marking them for later recapture (Lebreton et al., 1992). Age can then be determined upon subsequent recaptures. Size-frequency data compare the number of individuals within a given size class and associates those classes with particular ages. A review of this method and its efficacy on variety of herpetological models can be found in Halliday and Verrell (1988). It does however require a quantitative measure of the correlation between body-size, age, and the extent of variance. Moreover, it assumes that there is a clear association between age and size. However, many of these assumptions have come under scrutiny (Halliday & Verrell, 1988).

Currently, skeletochronology is the most reliable method to measure age in most vertebrate classes (Castanet et al., 1993). Castanet et al. (1993) describe skeletochronology as, "individual aging using the natural marks recorded in hard tissue, chiefly the skeleton, of growing animals." As long as these natural marks are acquired in a predictable "rhythm of known periodicity," then age can be gleaned by simply counting

the number of natural marks on the bone or other tissue (Castanet et al., 1993; Castanet & Naulleau, 1985; Meunier, 1988; Tucker, 1997). These marks form because of the uneven growth in tissue throughout the year. During favorable seasons, bone tissue is deposited at a faster rate than during unfavorable seasons. The consequence of this phenomenon is consecutive light and dark bands on the tissue. As long as those seasons of favorable and unfavorable growth are periodic, a researcher can estimate age. For reference, the bands that are formed during periods that do not allow for growth are defined as "lines of arrested growth" or "LAGs". These present as thin dark bands in the bone tissue. Bands formed during periods that are less favorable to growth are called annuli, and present as wider light colored bands. Those formed during favorable conditions are "growth zones" or "GZ's" and present as thick darker bands. Conformational studies must be run to assure that age correctly corresponds to LAGs and GZ's (Castanet et al., 1993; Castanet et al., 1996; Castanet & Naulleau, 1985; De Buffrenil & Castanet, 2000; Meunier, 1988; Trenham et al., 2000; Tucker, 1997). To accomplish this validation, several methods have been considered, including mark-recapture, artificial hibernation experiments, fluorescent labeling, and several others (Castanet et al., 1993). The use of natural indicators of age marks, particularly the use of yolk-scars has not been examined. That being said, the efficacy of skeletochronology has been confirmed in all vertebrate taxa where it has been applied (Bastien & Leclair, 1992; Castanet, 1987; Castanet & Baez, 1988; Castanet et al., 2004; Castanet et al., 1993; Castanet et al., 1996; Z. L. Clark & Zalisko, 2002; Coles et al., 2001; De Buffrenil & Castanet, 2000; El Mouden, et al., 1999; Halliday & Verrell, 1988; Jakob, et al., 2002; Scholz et al., 2010; Trenham et al., 2000; Tucker, 1997; Waye & Gregory, 1998; Zug & Rand, 1987; Zug & Glor, 1998).

One downfall of skeletochronology, however, is the possible formation of anomalous GZ's or LAG's (Castanet, 1987; Castanet et al., 1993; Meunier, 1988). False GZ's could theoretically form at any point where significant growth is possible. This means that even during unfavorable seasons of growth, if a large enough aberration in climate, creating proper conditions, were available, a small GZ could form in the middle of a LAG that would not coincide with a year mark. The opposite is also possible, when unfavorable conditions occur during a favorable season then an erroneous LAG may form. These unfavorable conditions might include temperature, lack of rain, starvation from lack of food, or other important life-events, such as reproduction. The extent of this problem may be limited however, because evidence suggests that cyclic bone production resulting in LAGs and GZ's may have a genetic endogenous control that is resistant to some of this environmental variability (Castanet et al., 1993).

Impact of stochasticity on age

The method of LAG counting can be applied to measure changes in life-history variation by sampling age-structure of different populations. As discussed before, variation in age structure can result from stochasticity in the environment (Benton et al., 1995; Boyce et al., 2006; Kaye et al., 2001; Lande, 1993; Morris et al., 2008; Oron et al., 2004; Penteriani et al., 2005; Saether, 1997; Facka et al., 2010; Peters et al., 2011). The major types of environmental stochasticity include variation in climate, food availability, flooding, drought, fire, and habitat availability. Flooding stochasticity, in particular, drastically alters a landscape, which can change the availability of habitat, food, water, thermoregulation sites, and breeding sites. Flooding may occur on a semi-predictable schedule. However, these events can act unpredictably as well, especially when

considering anthropogenic influences on the environment. Floods often happen predictably during seasons of heavy rainfall. The exception to this is when floods are modified by the presence of dams. Such alteration of natural rivers makes the timing of flood events much more unpredictable (Hampton & Ford, 2007). Also, the extent of floods are unpredictable, particularly in these regulated systems (Ford & Lancaster, 2007; Hampton & Ford, 2007).

Flooding semi-periodically inundates these areas with water that is often deep enough to drown most organisms living on the floodplain floor. Floods create a highly dynamic system; river channels are constantly changing shape and creating new habitats by knocking down trees and washing out old debris. Flood water can take weeks to recede and forms many microhabitats and mesohabitats in the process. Floodplain systems fluctuate in amount of water on a monthly, yearly, and sometimes longer cycle. Drought often strikes unpredictably, creating unseasonably dry weather, which drastically influences the animals living in these systems. Many of the species living in floodplains depend of flooding to accomplish their life-cycles. This is particularly true of amphibians and fish, which depend on water on a seasonal basis for reproduction. Anthropogenic effects exacerbate the stochasticity and degree of flooding.

As a consequence of these variables, organisms must be able to deal with fluctuations in food, water, habitat, and mortality rates, all of which strongly influence life-histories. Therefore, floodplains, particularly those associated with bottomland forest, are an interesting system for measuring life-history variation in organisms because they can be highly stochastic and are a source of rich diversity and abundance of many taxa of animals and plants.

More stable wetland habitats such lakes and ponds are not very stochastic in nature. These systems rarely flood outside their banks and their micro and mesohabitats remain the same for much longer periods of time. Typically these systems hold water year-round, allowing organisms to easily complete their life-cycles with a dependable source of water and food. Therefore, one expects to find more stable or less variable life-history strategies being utilized in stable environments.

Modeling life-history variation

A variety of vertebrate and invertebrates have been used to model life-history variation, ranging from fruit flies and daphnia to birds and reptiles (Beuchat & Ellner, 1987; Leroi et al., 1994; Martin, 1987; Spitze, 1991). Fewer studies, however, have used snakes to model such theories, though they make ideal candidates for such research. Snakes are common and important members of most wetland ecosystems. They demonstrate a wide range of life-history variation because they are ectothermic, indeterminant growers, and many are viviparous (Shine, 2003; Shine, 2005). Snakes are also convenient models as they are abundant, easy to maintain in captivity, are diverse in their niches, and have reproductive and growth traits that are easily measured. In addition, snakes fill many roles within the food web

Study species

Several studies have examined the life-history characteristics of the western ribbon snake *Thamnophis proximus*, the focal species of this study (Conant, 1965; Lancaster & Ford, 2003; Rossman, 1962; Rossman, 1963; Rossman et al., 1996; Tinkle, 1957). A summary of some early findings can be found in Rossman et al. 1996. This species is native to the central and southern United States and Mexico (Rossman et al.,

1996). The ribbon snake uses a variety of aquatic habitats including marshes, ponds, lakes, rivers, creeks, and sloughs. This makes this species an excellent subject for examining the effects of stochastic and stable ecosystems on life-histories, as many of these aquatic systems flood regularly disturbing the surrounding environment while others are relatively more stable (Ernst & Ernst, 2003; Rossman et al., 1996). Seasonality in behavior also makes these snakes strong candidates for age-structure studies through use of skeletochronology. They tend to become dormant during colder months of winter, especially in temperate climates, allowing for strong LAG formation (Ernst & Ernst, 2003).

As indicated, some of the aquatic habitats these snakes inhabit are highly stochastic. Particularly, regulated waterways and their associated bottomland forests may undergo drastic changes in their landscapes during a flood event thus altering potential feeding, thermoregulation, parturition, and denning sites for snakes inhabiting them. In addition, floods, or lack thereof, predominantly in regulated waterways, can change the food availability for ribbon snakes that prey mostly on amphibians and fish (Carfagno & Weatherhead, 2009; Ernst & Ernst, 2003; Hampton & Ford, 2007; Rossman et al., 1996). All these factors and the additional risk of drowning and/or physical damage during a flood event elevate the risk of mortality in these types of habitats. Theoretically, we would expect to see a difference in the suite of life-history characteristics in ribbon snakes populating different types of habitats. Stable bodies of water, specifically lakes and ponds in higher elevation areas, do not experience such drastic changes and offer snakes more stable food sources, habitat availability, and may therefore potentially lower the risk of mortality in western ribbon snake populations occurring in these areas. Thus,

this project was conducted to compare life-history traits of ribbon snakes found in each of these habitats with the goal of trying to elucidate differences that would support the current theory of life-history evolution.

I predict therefore that life-history traits of western ribbon snakes living in stochastic floodplains should exhibit "fast" life-history strategies, where energy is diverted to fast growth rates, larger body-sizes, and higher annual fecundity compared to more stable environments. Consequently, they should also have shorter lifespans, with younger mean ages for the population.

A summary of the western ribbon snake's life-history is available from populations of this species across the Southeast United States. Tinkle, Clark, Rossman, Conant, and Lancaster et al. all have published extensively on this species directly (Clark, 1974; Conant, 1965; Lancaster & Ford, 2003; Rossman, 1963; Tinkle, 1957).

Study sites

To represent a stochastic environment, the Old Sabine Bottom Wildlife Management Area in Smith County, Texas was selected. It is a 2,087-hectare reserve run by the Texas Parks and Wildlife Department and is located within the floodplain of the Sabine River. It is predominantly bottomland hardwood forests supporting a large population of *Thamnophis proximus*. It is known to support a diverse array of amphibian species, to which ribbon snakes are known to feed most heavily (Hampton, 2008). Species of amphibian include the *Lithobates sphenocephala, Lithobates catesbeianus, Lithobates clamitans*, *Hyla versicolor, Hyla chrysoscelis, Hyla cinera, Pseudacrics crucifer, Gastrophryne olivacea, Gastrophryne carolinensis, Ambystoma texanum, Ambystoma opacum, Anaxyrus woodhousii, Acris crepitans, Siren intermedia* and

potentially others. In addition to supporting amphibian species, this floodplain also retains many fish species that become isolated in pools as the Sabine River floodwaters recede. Many of the smaller species, particularly *Gambusia affinis*, are also major prey items for western ribbon snakes. Lastly, this site plays host to several lizard species that are potential prey for ribbon snakes. These include *Scincella laterale, Eumeces fasciatus*, and *Eumeces laticeps*. As might be expected, several potential predators have also been documented here including *Sus scrofa*, *Canis familiaris*, *Procyon lotor*, *Didelphis virginiana*, *Memphitis memphitis, Spilogale putorius*, *Buteo spp.*, *Strix spp.*, *Egretta spp.*, *Ardea spp.*, *Turdas migratorius*, *Cathartes spp.*, *Corvus brachyrhynchos*, *Agkistrodon piscivorus*, *Lampropeltis getula*, and *Alligator mississippiensis*.

The study area typically floods during winter months when the area receives its heaviest rain. Rare spring and summer flooding does occur when unseasonably wet weather arrives or water is being released from reservoirs upstream. During a typical flood event, most ground surface in the floodplain is inundated, and can be several meters underwater for weeks afterward. Often, most amphibians breed soon after the area has flooded. Prey items, particularly tadpoles and fish, often get trapped in shallow pools as floodwaters recede. It has been documented that ribbon snakes take advantage of this situation, and aggregations can be found feeding around these pools (Ford, pers. comm.; pers. obs.). During the hottest months of the year, very little water can be found in this site, except for in some of the largest oxbows of the river.

To represent a stable environment, a large series of mostly permanent ponds at Camp Tyler was selected. Camp Tyler is 142-hectare property also located in Smith County, Texas on the Lake Tyler Reservoir, which is a large permanent body of water.

Most of the ponds on the property maintain water year-round and are surrounded by mixed forest and grassland/pasture. The property is privately owned and hosts an educational camp that runs year-round. It can have several hundred visitors over the course of the year, particularly during the field season. Densities of snakes at this site are unknown, but we predict them to be significantly lower owing the smaller area of this site. This area also has abundant amphibian populations, particularly in the more ephemeral pools and smaller permanent pools lacking fish. Species documented here include: *L. clamitans, L. catesbeianus, H. versicolor, H. chrysoscelis, H. cinera, P. crucifer, A. crepitans, A. tigrinum, G. carolinensis, A. woodhousii, S. intermedia*. In addition to these amphibians, many of the permanent ponds/lakes are stocked for fish or have permanent populations in them; these include: *Lepomis spp., Ictalurus spp., Gambusia affinis*, and potentially others. This site also has large populations of lizard species including *S. laterale, E. fasciatus*, and *E. laticeps*. Several known and potential predators live in the area including raccoons, opossums, striped and spotted skunks, red-tailed hawks, red-shouldered hawks, barred owls, egrets, herons, robins, vultures, crows, cottonmouths, and speckled kingsnakes. This system also receives most of its rainfall in winter and early spring, which replenishes those ponds/lakes that maintain permanent water, while also serving to form many temporary pools. Most of the amphibians breed during this period. By the end of June most all of the temporary pools have evaporated. Ponds form in both open grassland habitats as well as forested ones.

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Chapter Two

Introduction

Life-history traits often vary in response to environmental variables (Bardsen et al., 2011; Bronikowski, 2000; Murphy, 1968; Stearns, 1977; Stearns, 1989; Wilbur & Rudolf, 2006). These traits differ in a variety of ways depending on the environmental stressor affecting the individual. Measuring age-structure differences between populations can be a valuable method for detecting this variation in life-history traits between populations with separate environmental stressors (Boyce et al., 2006; Bruce et al., 1996; Esteban & Sanchiz, 2000; Ford & Seigel, 1994; Leskovar et al., 2006; Miaud et al., 1999; Morrison et al., 2004; Scholz et al., 2010). Age-structure of a population is directly related to the life-history of individuals. If a shift occurs in life-span, age of reproduction, or growth rate, all of which are important life-history traits, it would be clearly evident in the age-structure of the population. The average age and distribution of individuals in age classes would be altered.

Many environmental factors influencing populations have been examined for correlation to variation in age-structure and other life-history traits. These environmental factors include elevation, drought, climate, and fire (Bardsen et al., 2011; Esteban & Sanchiz, 2000; Franklin et al., 2000; Jakob et al., 2002; Kaye et al., 2001; Miaud, Guyetant, & Faber, 2000; Morrison et al., 2004; Rohr, 1997; Sagor et al., 1998; Sherman & Runge, 2002; Sumerford et al., 2000; Willson et al., 2006; Winne et al. 2006). Only a

few studies have looked at the influence of flooding on life-history traits (Blouin-Demers & Weatherhead, 2007; Brown & Shine, 2007; Madsen & Shine, 1999b; Naiman & Decamps, 1997). Few of these studies have examined the influence of flooding, specifically, on reproductive traits and none have used age-structure to measure any life-history variation occurring in flooded habitats (Madsen & Shine, 1996b; Madsen et al., 2006).

Floodplains are highly stochastic systems that are expected to cause organisms within them to show considerable difference in life-history traits compared to those in stable environments. Flooding or lack thereof can trigger fluctuations in food availability, micro-habitat availability, and mortality from drowning or desiccation (Hampton & Ford, 2007; Madsen & Shine, 1999a; Madsen & Shine, 1999b; Naiman & Decamps, 1997). Because of these factors, it's expected that individuals in such environments should live comparatively shorter lives than those found in stable aquatic habitats, such as lentic systems. Individuals should grow faster or be on average, younger per body-length than those in stable systems. Comparisons of mean age or mean body-length can help elucidate any differences related to flooding patterns.

One group of animals that has historically been studied for life-history variation is snakes. Snakes are considered good models for studying patterns life-history evolution because many of their traits are highly plastic/adaptable and they are abundant in many ecosystems (Ford & Seigel, 2006; Seigel & Ford, 2001; Shine, 2003; Shine, 2005). The western ribbon snake (*Thamnophis proximus*) is a particularly good model for measuring life history variation caused by flooding because it makes use of a broad range of aquatic and semi-aquatic habitats, including stable lentic systems and stochastic floodplains

(Ernst & Ernst, 2003; Rossman et al., 1996). In addition, these snakes feed most commonly on a variety of amphibian prey, whose life-histories are dependent on the availability of water (Ernst & Ernst, 2003; Ford & Hampton, 2009; Rossman et al., 1996).

Unfortunately, there are only a limited number of methods for aging individual snakes in order to estimate the age-structure of a population. The most common methods for estimating age of individuals are mark-recapture data, size-frequency data, and skeletochronology (Halliday & Verrell, 1988). Mark- recapture is the most effective but labor-intensive method and can take years to accumulate an appropriate sample size. Size-frequency data is the least effective method and has come under a large amount of criticism due to its assumptions about growth (Halliday & Verrell, 1988). When applied correctly, skeletochronology has been applied to most vertebrate groups with a fair amount of success (Castanet, 1987; Castanet et al., 2004; Castanet et al., 1993; Meunier, 1988). It can give an estimation of age for individuals within a population. These data are then used to estimate the age structure of the population.

Nonetheless, several drawbacks are associated with the use of skeletochronology for estimating age of individuals. Besides being a difficult method to use and interpret, it requires validation and intensive labor (Castanet et al., 1993). Validation can be difficult to accomplish without the use of long-term studies and recaptures. The need for validation of skeletochronology is essential if useable age data are to be gleaned. The fact that skeletal banding can be caused from several non-cyclic, non-predictable factors can lead to erroneous conclusions about an individual's age. Additionally, validation for many taxa and in several climates has not been performed. This is also true for organisms

in East Texas, a near sub-tropic climate where the formation of false growth marks may occur. A few papers have discussed the use of skeletochronology in sub-tropic climates, but have found only partial success (Esteban et al., 1996; Morrison et al., 2004).

Factors that might lead to false growth marks in the western ribbon snake in East Texas include starvation, irregular aestivation and hibernation patterns, shedding, and reproduction. It has been shown in garter snakes of the genus *Thamnophis* living in more temperate climates of the upper Midwest and Canada, that growth mark formation is in fact periodic (Waye & Gregory, 1998). These individuals are known to form a single LAG in winter and a single GZ during the rest of the year. In sub-tropical areas such as East Texas, this association has still to be validated.

Therefore, the objectives of this study are: (1) examine the age-structure of populations in areas of non-flooding and those that are subject to flooding and (2) validate the use of skeletochronology for estimating the age of individuals snakes in East Texas. In addition, a comparison of a site with many stochastic impacts with a more stable site will be used to examine which factors might be involved if validation is difficult. This will be accomplished by first comparing mean growth mark number and mean body size in two sites, one a floodplain and the other an upland lentic site. Several validation methods will be applied including use of yolk-scarred neonates, comparisons to a growth curve of western ribbon snakes estimated by Clark (1974) using mark-recapture data, and monthly capture rates. Lastly, climate variation will be analyzed to confirm stochasticity, predict surface activity of snakes, and compare this activity to potential variation in growth mark formation. The monthly number of floods will measure stochasticity of the floodplain site.

Methods

Study sites

To represent a stochastic environment, the Old Sabine Bottom Wildlife Management Area (OSBWMA) in Smith County Texas was selected. It is a 2,087-hectare reserve run by the Texas Parks and Wildlife Department and is located within the floodplain of the Sabine River. The habitat is predominantly bottomland hardwood forest supporting a large population of ribbon snakes. Flooding typically occurs in the winter months, and completely inundates the bottomland forest. As waters recede, pools form, trapping a large amount of potential prey items for ribbon snakes, particularly amphibians and fish. Most of these ponds will dry up by the end of the summer (Ford & Hampton, 2009). The likely causes of mortality for western ribbon snakes in this site are from predation, desiccation/starvation, and stochastic events.

To represent a stable environment, a series of ponds and lakes at Camp Tyler were selected. This site is also located in Smith County, Texas. Most of these ponds maintain water year-round and are surrounded by mixed forest and grassland/pasture. Neighboring the property is Lake Tyler, a reservoir of the Sabine River. The property is privately owned and hosts an educational camp that runs year-round. It can have several hundred visitors over the course of the field season, when the field data were collected, which may have some impact on snake abundances. Theoretically food is available year-round from a variety of amphibian, reptile, and fish prey. The site does not flood and therefore likely provides permanent habitats for ribbon snakes. The predicted causes of mortality for ribbon snakes are anthropogenic disturbance or predation from a large pool

of potential predators. This mortality, however, is predicted to be less than that from stochastic events.

Specimen collection

Cover objects (tin/boards) were placed at both study sites in areas of high ribbon snake activity, including feeding sites, thermoregulation sites, and refuge sites. These objects were checked January 2010 to April 2011 at all times of day to prevent bias, as their daily activity snakes may shift throughout the year. Both sites were visited at least once a week, but often more. In addition, specimens were collected from visual surveys in the field as well as on the road.

A total of 79 animals were captured and sampled between both the OSBWMA and Camp Tyler, 69 of these individuals were identified as western ribbon snakes, *Thamnophis proximus*. When captured, individuals were processed in the field and released immediately. Snout-vent length (SVL), weight, stomach contents, body condition, reproductive status, and sex were recorded for all individuals. One to two centimeters of tail tip were clipped and stored in 95% ethanol. The tail was then clotted using styptic powder. The ventral scales were clipped with an individual ID number to keep track of recaptures.

One western cottomouth, *Agkistrodon piscivorus*, four yellow-bellied watersnakes, *Nerodia erythrogaster*, four broad-banded watersnakes, *Nerodia fasciata*, and one Texas brown snakes, *Storeria dekayi* were also sampled as a comparison and validation to ensure growth marks were not unique to ribbon snakes in East Texas. Two ribbon snakes from Chichaqua Bottoms, Polk County, IA were also sampled to compare individuals from East Texas to individuals from a temperate region. Four female and four

male captive bred ribbon snake neonates were also measured to obtain data from individuals of a known age.

Processing

Tail tips were brought to the University of Texas Health Science Center Tyler (UTHSCT) for further processing. Immediately before paraffin embedding, tissues were soaked for 3 hours in a solution of 3-5% nitric acid in order to decalcify the bone material. Standard paraffin embedding protocol was used. A standard paraffin microtome was used to make 10 μm thick sections. Four to ten sections per individual were mounted on slides. After drying, sections were stained using hematotoxylin and eosin for 3 minutes each, using standard methods.

Counting

Age marks were recorded under a light microscope, and images were captured using a Nikon mounted camera. Re-assessment of any individual due to confusion about number of marks was performed from images. A count consisted of the number of complete growth mark series in the clearest section of bone. Multiple magnifications (40-400x) were used to get the clearest read.

Several precautions were taken to prevent counter bias of growth marks. First, before counting, a set of growth marks was defined as a wide growth "zone" that appeared darker and either or both an annulus and/or a line of arrested growth (LAG) (Castanet et al., 1993). Annuli present as lighter colored bands in bone tissue where there is slower growth in the bone tissue. A LAG represents an area of completely suspended bone growth and is observed as very dark thin band next to an annulus or growth zone (Castanet et al., 1993). Second, the identities of the individuals were blinded from

counter and tail tips were then selected at random. This ensured that the counter would not be able to bias the count by accounting for body size or previous knowledge of the individual. Third, a second naïve counter was given several randomly selected individuals to compare the precision of counting. The respective numbers of growth marks from each counter were compared for consistency. During the counting process, if any individual's sections were difficult to count and the number of growth marks was different across these sections, then the average count between all the sections was used.

Data assessment

For analysis, an individual's count was put into an ecologically relevant class. These classes were 0, 1, 2, 3, and 4 or greater. Individuals in class 0 are assumed to be newborns of the year and should still have prominent yolk scars. Class 1 are individuals who have survived their first winter, should have one complete growth mark set, should not be sexually mature (male or female), and may still have yolk scars. Class 2 is assumed to be the youngest age individuals could be sexually mature (Clark, 1974; Tinkle, 1957). Class 3 is individuals that become sexually mature in their third year of growth (Clark, 1974; Tinkle, 1957). The last class should consist of individuals that are all sexually mature and have already had one mating attempt. Two-way analyses of variance (ANOVA) were performed to compare each respective group to see if mean age/growth marks and mean length was different between groups. Additionally, regressions of body length to age/growth marks were performed as a method of validation.

Validation

Several methods were used to validate skeletochronology methods in East Texas including recaptures, yolk-scarred neonate comparisons, growth curve comparisons of known-age individuals, and analysis of surface activity patterns.

First, an effort to find recaptures from 2010 was made from February 2011- April 2011. Marked individuals were re-sampled to ensure the addition of only new annulus/LAG (no recaptures, however, were collected). Secondly, during that same period, tail tips were collected from juveniles with prominent yolk scars. These individuals are expected to have a single annulus and/or LAG because they theoretically should have gone through a single hibernation. They were compared to known age 0 individuals as a reference, which should have no LAG's or annuli. Thirdly, using growth curves from Clark (1974), data were compared to expected values from Clark's population of *T. proximus* in Brazos County, Texas (Clark, 1974). Clark (1974) released several marked female neonate ribbon snakes in Brazos County, Texas. Upon subsequent recaptures of these individuals over the next two years, he was able to plot growth trajectories based on body-length (SVL) to month recaptured after the release date. A mean age to body-length trajectory for females was plotted. Subsequently, age could be predicted given the body size of the snake and the month in which it was captured. Criteria for comparison of females from my data consisted of females ≤520 mm snout-vent length (SVL) or females that exhibited 0-2 complete growth marks (females greater than 520 mm SVL, Clark considered adult) from either site. Also, females that had growth marks that corresponded to the predicted age on the trajectory were compared to see if they same predicted body-length. Unfortunately, no measure of variation was

included with Clark's trajectory (1974), so and variation from the mean can only be compared subjectively.

Lastly, another attempt to validate the one-year one-band hypothesis was to further understand the circannual surface activity cycles of ribbon snakes. This was used to assess the presence of a single hibernation event yearly and the absence any aestivation events. This underlying assumption of skeletochronology requires the formation of a single annulus/LAG and that no extraneous bands could be formed. Capture records from the Texas A&M Natural History Collection for the entire state of Texas, from Clark (1974), Ford (unpub. data), Hampton (unpub. data), and my capture records were analyzed to find capture rate per month (number of snakes collected per month). Capture rate was used a proxy for surface activity, as it assumes that snakes hibernating or aestivating will be unavailable for capture due to hiding. All data were using snakes captured in the state of Texas. Clark's population of snakes was from Brazos County, Texas. Hampton, Ford, and my data were all collected from the OSBWMA, Smith County, Texas. All collections sampled year-round. Collection data was compared to climate and stochastic data that might influence surface activity and therefore growth mark formation. A comparison of average rainfall, average monthly highs, and average monthly lows were compared to capture records. Data are compiled from the United States Geological Survey and the National Weather Service. These factors give a better understanding of favorable surface condition to allow snake activity and growth, as they are associated with factors that readily cause mortality in ribbon snakes. Factors that cause mortality are hypothermia, hyperthermia, and desiccation.

Measure of stochasticity

To give evidence that aquatic micro-habitats shift, particularly in the OSBWMA during the year, and that the OSBWMA is a stochastic system due to flooding, monthly flooding events were recorded from 1996-2010. These data were also pooled from the USGS Water Survey using the river gauge on the Sabine River near Mineola, Texas. Floods were defined as any stage measured past the USGS defined flood stage.

Results

Growth mark counts

All species sampled showed some growth marks (Figures 1 a-e). The cottonmouth sample in particular showed distinct bands. Moreover, the two ribbon snakes collected in Polk County, IA had very well defined growth marks, as would be expected from individuals living in more temperate climates. Banding can be seen in individuals from both study sites and from captive-born neonates (Figure 2 a-c).

A sample of 11 specimens were collected from Camp Tyler, three females and eight males. Body size and number of growth marks did not vary substantially (Table 1). Another male ribbon snake, presumed to be an adult, was captured and measured but growth marks could not be measured from its tail sections.

A larger sample of 51 individuals was sampled at the OSBWMA, twenty-five females and twenty-six males. An additional five males were captured and measured from this population but sections had indiscernible growth marks.

Growth marks in each population can be summarized as follows. Of the three females captured at Camp Tyler, each fell into a separate “age/growth mark” class, 1, 2, and 3. Males within this population showed more variation with two individuals showing

1 growth mark, one showing 2 marks, one showing 3, three showing 4, and one showing 7 marks. All means are reported as mean ± the standard deviation The mean growth marks for females at Camp Tyler was 2±1, and for males 3.3± 2. The individual in this population with most growth marks was a male with 7 growth marks. At the OSBWMA, a clearer distribution of growth marks can be seen. Of the females captured at the OSBWMA, three showed no growth marks, two showed 1 growth mark, three showed 2, one showed 3, sixteen individuals showed at least 4 growth marks. The females with the most growth marks, showed 11. The males followed a similar distribution. Two males showed no growth marks, two showed 1 mark, three showed 2, four showed 3, and fifteen showed 4 or more growth marks. The most marks recorded on any male in this population were 9. A summary of the mean growth marks and mean SVL of snakes captured is shown in Table 1. The percentage of ages of these marks between each treatment group is also shown in Figure 3.

There were no differences in number of growth marks between any of the four groups tested, Sabine males, Sabine females, Tyler males, and Tyler females ($F_{sex,df=1}$= 0.16, p= 0.689; $F_{site,\ df=1}$= 2.69, p= 0.106). Between Sabine males and Sabine females there was also no significant differences detected in mean growth mark number (p=0.54). Similarly, when snout-vent-length was analyzed between groups, nothing significant was detected ($F_{sex,df=1}$= 0.70, p= 0.407; $F_{site,\ df=1}$= 0.53, p= 0.469)

Regressions of growth marks to body-length were performed to examine if a correlation exists between these two traits (Figure 4). It's expected that number of growth marks should increase with body-length until ribbon snakes reach adulthood, where they should then taper off. All four linear regressions yielded weak correlations, as

demonstrated by their R^2 values (Sabine Females: R^2= 0.180, Sabine Males: R^2= 0.163, Camp Tyler Females: R^2= 0.553, Camp Tyler Males: R^2= 0.133).

Validation

Yolk scars

Two individuals of *T. proximus* coming out of hibernation collected in March of 2011 still had prominent yolk scars and were at sizes close to newborn neonates in lab (Figure 5 a-b). Additionally, neonates from the OSBWMA are documented to give birth July- early September (Figure 5c) (Lancaster & Ford, 2003). I therefore concluded these snakes to have been born the previous summer and had completed their first hibernation. Both snakes did indeed have a growth zone and an annulus although it was only distinct in one individual. Both individuals lacked a distinct LAG, indicating that a complete arrest of bone growth did not occur.

It should be noted that even though individuals with recorded yolk scars showed single growth marks, similarly sized individuals, who should also have yolk scars that were not recorded, collected in April 2010, showed no growth marks. This stands in contradiction to those individuals with yolk scars. These individuals should still have yolk scars but cannot be newborns of the year. The three females collected at the OSBWMA showing no growth marks were collected in April 2010, as mentioned earlier, measured 33.1, 28.5, and 20.5cm. In addition, individuals of three other snake species that had prominent yolk scars were sampled to confirm this method of validation. These snakes showed growth marks with varying completeness and clarity, the best being in *Storeria dekayi*.

Clark's growth curve

In a previous mark-recapture study of neonate female ribbon snakes, Clark (1974) was able to estimate a growth curve with variation for individuals up to two years of age. In this study, eighteen females, 15 from the OSBWMA and 3 from Camp Tyler, were compared to Clark's growth curve and variation (Figure 6). If one examines Clark's mean line plus the major variation around the mean at a given month, they would notice that while some individuals sit within the expected range nicely, several individuals sit widely outside their expected range. The first group of individuals that should be mentioned are those that are less than 520 mm but show substantially more growth marks. Six individuals fall into this category. The smallest of them was 310 mm showing four growth marks and was captured in April. The largest was 493 mm and showed eight growth marks captured in April. Clark's growth trajectory shows a tapering off of growth after May of a snake's first year. This may be attributed to individuals slowing growth at a smaller size than others. Even still, the two smallest individuals showing two growth marks measured 310 mm and 360 mm. These are very small sizes compared to Clark's mean for female adults at two years or older.

The second group consists of individuals who show 1 growth mark but are drastically larger than the mean predicted size for a snake captured in that month. There are two individuals in this group. The first is a 585 mm female captured in April and the other is a 620 mm female captured in May.

The last group consists of an individual that should have 2 growth marks but is drastically smaller than what would be predicted for an individual captured in that respective month. This female was 240 mm captured in May. Clark's average for a

female captured in May of its second year is 510 mm, over twice the length of this captured female.

Climate, local environmental factors, and capture rate

Rainfall

Annual rainfall in East Texas (Tyler) is 122 centimeters. The majority of this rain occurs in the fall/winter months of October, November, and December (14, 13, and 13 cm) when the air temperatures are on average lower. Higher rainfall on average also occurs in May (12 cm). The lowest rainfall tends to occur in the hottest summer months of July and August (6 and 7 cm). A corresponding drop in capture rates are noted from Clark, Ford, Hampton, and Chamberlain during the months of July, August, and September, potentially due to the lack of rainfall in these months. A drop is also noted from the Texas A&M collection, however the drop is not distinct until September.

Temperature

The yearly average high temperature in East Texas is 25˚C, and the yearly average low is 13˚F. The coldest months of the year, January and December have average highs of 15 and 15˚C and average lows of 3 and 4˚C respectively. The warmest months of the year, July and August, average 34 and 35˚C for highs and 23 and 22˚F for lows respectively. Rossman et al. suggest that in order for *T. proximus* to be active, air temperatures must be consistently >15˚C (Rossman et al., 1996). Hibernation, they suggest, might be intermittent in southern localities, and that if unseasonably warm weather occurs, some feeding can occur (Rossman et al., 1996). However, Clark, Ford, Hampton, and Chamberlain's observations did not agree with these findings (Clark, 1974). Instead, it appears that very little surface activity occurs November through January. Temperatures above 15˚C on average stop in November and do not return until

February. A similar trend is found the Texas A&M Collection. Their three lowest months for captures in the entire state were also December, January, and February.

Average highs in late summer also suggest unfavorable daytime temperatures for active ribbon snakes, which are a diurnal species (Rossman et al., 1996). July and August have the highest average temperatures and lowest monthly rainfalls. Having lower capture-rates during dry spells in Brazos County, Clark's data give further evidence for summer inactivity during dry spells (Clark, 1974). Furthermore, there is little evidence for nocturnal activity of ribbon snakes in Texas, though it has been documented elsewhere, even though nightly temperatures may be more favorable for activity (Rossman et al., 1996). While having higher captures rates during July and August compared to January and December, Ford, Hampton, and Chamberlain all had decreased captures compared to April, May, and June, suggesting that at least a portion of populations in East Texas have decreased activity during these months. This trend is not seen throughout the entire state of Texas, however. The Texas A&M collection does not see a distinct drop in capture rates until September, where they stay low until the following March. This suggests that water availability and not temperatures is what is causing the distinct decrease in capture rates for East Texas.

A summary of the climatic data can be seen in Figures 7a and 7b.

Measure of stochasticity

Stochasticity of the OSBWMA was estimated by the monthly rate of floods of the Sabine River in the area. Clear trends can be noticed from this data (Figure 8). First, December through April tend to have more floods than the other months. This is expected given the highest rainfall occurs November and December with ample rainfall also

occurring in January, February, March, and April. Secondly, flooding during the hottest and driest month of the year, August, is extremely rare. Floods during other hot summer months occur with a slightly higher frequency but are still comparatively rare to the rest of the months. Flooding appears to be fairly predictable in the winter and spring but can be rare and/or unexpected during hot summer months. While flooding in general can define a system as stochastic, rare unseasonable flooding amplifies this stochasticity.

Discussion

I conclude that skeletochronology is not an effective method for aging individual ribbon snakes in East Texas, based on several non-traditional validation methods including yolk-scars on neonate snakes, comparisons to known growth curves of other populations, and understanding surface activity based on climate and capture rate. All combined methods of validation indicate that skeletochronology is a poor indicator of actual age in East Texas.

Many papers have sought to validate skeletochronology in their model organisms, particularly when unsure about the natural history of that organism (Castanet et al., 2004; Castanet & Naulleau, 1985; Coles et al., 2001; De Buffrenil & Castanet, 2000; Meunier, 1988; Morrison et al., 2004; Tucker, 1997; Waye & Gregory, 1998; Zug & Rand, 1987). In temperate climates, the use of skeletochronology has been well substantiated. Few studies, however, use skeletochronology in warm temperate to sub-tropic climates and therefore validation attempts on animals living in these areas are limited (Esteban et al., 1996; Morrison et al., 2004). Most all validation attempts look at rate of bone production and LAG formation using fluorescent markers or using mark-recapture data. Neither of these methods was available in the current study, so alternate methods were used. While

it is preferable to use these traditional forms of validation, when time is restricted, these alternate forms of validation are effective for skeletochronology.

The use of yolk-scars was not able to conclusively validate that ribbon snakes always added a single set of complete growth marks in single year of growth. Snakes with yolk scars did have growth marks and annuli like predicted. However, similarly sized individuals that should theoretically also have yolk scars, though not documented, sometimes, not always, had no annulus or had too many complete growth marks. Additionally, several individuals measured with more than nine growth marks. Yolk scars have been used elsewhere for aging purposes (Neill, 1962; Smith, 1947; Wynn & Zug, 1985). To my knowledge, however, this is the first application of yolk scars to try to validate skeletochronology. Further trials must be run to truly understand the efficacy of yolk-scars to validate this method.

Weather and flooding patterns suggest mixed results as to the predictability of surface activity of ribbon snakes. Surface activity, as mentioned earlier, is unpredictable from year-to-year, thus the corresponding growth mark formation is also unpredictable. It appears that hibernation is a fairly predictable phenomenon that lasts a minimum of two months in East Texas. Temperatures below 15ºC are maintained on average December and January. This should easily allow for the formation of an annuli or LAG. However, the data also suggest that some but not all individuals aestivate during the unfavorable summer months in Texas. This means that only some of the population may put on two growth marks a year, while others may only put on one. This is evidenced by unfavorable high averages, increasing chances of hyperthermia, and low rainfall, increasing the chance of dessication and starvation due to food shortage. In order to avoid this increased

chances of mortality, some snake species aestivate (Willson et al., 2006). Given that all five collections should decreased captures during the months of highest temperatures and lowest rainfall, I conclude that some ribbon snakes in East Texas do in fact hibernate. Further compounding the problem is the fact that even though some may aestivate during highly unfavorable conditions, this period may not be long enough to substantially reduce bone production to create an annulus or LAG, therefore it can't even be accounted for with any degree of certainty. Also, partial LAGs or split LAGs were sometimes detected. Some individuals may better handle this aestivation than others and therefore individual-specific banding occurs rather than population banding, as is assumed by skeletochronology. Some individuals that stay active during these unfavorable seasons might physiologically have to do so. Gravid females, which typically do not feed near the end of their gestation and therefore are uninfluenced by a lack of summer food, might need to stay active to properly thermoregulate the young they carry inside. Males on the other hand, which have already completed their matings before the hot and dry late summer months, may go into aestivation to avoid risk of desiccation and starvation.

Linear regression coefficients of growth marks to snout-vent length also indicate a weak relationship between a snake's length and how many growth marks they have accrued. As indeterminant growers that continue to gain in body size throughout their lifetime, its predicted that the correlation between age and body size should be high. The low correlations indicate one of two things. First, these weak correlations could show that the age-body size relationship is not true in this species, previously shown in other species (Halliday & Verrell, 1988). Otherwise, weak correlations could indicate that growth marks do not actually represent age. The regression coefficient for Camp Tyler

females suggests a strong correlation, however the regression line was calculated from only three individuals

Other issues of validation of skeletochronology in East Texas stem from comparisons with the female growth trajectory provided by Clark. Clark estimated growth curves for marked female neonate snakes released into the field through the first two years of growth. My data was compared to this known age to body size relationship as a form of validation. While it should be said that some individuals plotted correctly within their expected range, many others did not. Two conclusions may be drawn from these comparisons. The first conclusion requires the assumption that growth marks in these populations do in fact represent a periodic cycle allowing proper measurement of age. With this assumption, one is left to conclude that ribbon snakes are widely variable in body length at a given age. Some individuals showed hardly any growth after two years, while others showed enormous amounts of growth within the first nine months of their life. The amount variation seen seems improbable. In order for a two-year-old snake to have the same body length as the average neonate in its first summer, it would require that animal to completely starve itself for two years, which would almost certainly kill that animal. Similarly, for a snake to be one of largest individuals in the population after only nine months of life, 2-3 of which were spent in hibernation also appears unreasonable. It appears that age could be predicted with limited success, thus this method of validation should be explored further in other species where data on growth curves is available.

Methodology is also a concern when considering accuracy of measurements for skeletochronology. Count errors seem to be inevitable to do unclearly cut sections,

broken bone fragments, and under and over stained sections. Some of these issues were mitigated by the use of the average count from all the sections used for a given individual. Even though bands may have been present, they could often be difficult to count due to some of these misleading factors. Several of these issues may be resolved by improving techniques of paraffin embedding, sectioning, and staining. A weakness of the preservation method used in this study was immediately preserving tail-tips in ethanol. First preserving tissues in formalin to stiffen them, and then transferring them to alcohol might improve clarity of sections. This is because stiffer objects are easier for the microtome to section. A variety of other preservation, sectioning, and staining methods are available for skeletochronology. These methods should be explored to discover the most effective combination of factors. Some variation in number of growth marks cannot be accounted for, including the destruction or remodeling of bone matter and its corresponding bands. This issue is addressed extensively by Castanet (Castanet et al., 1993). He gives several techniques for detecting and accounting for bone remodeling. While these issues doubtless play a role in skewing our results, we were unable to account for their presence.

Skeletochronology is a useful tool and it may still be applied to populations in East Texas. It is important to remember that snakes here predictably go into hibernation on a yearly basis resulting in the formation of a LAG or annulus. If one were able to account for the environmental-noise caused by aestivation or other factors, this method might could be applied in East Texas or other sub-tropical climates. Furthermore, other species of snake or reptile/amphibian show promising results. Cottonmouths, for instance, showed strong banding. These snakes also make a strong candidate for use with

skeletochronology because they are documented to stay active during hot summer months by switching their diet to non-amphibian, non-fish prey such as rodents thereby eliminating the threat of starvation from lack of water (Ford, pers. comm.). Additionally, its larger body-size compared to ribbon snakes may help prevent desiccation. These factors combined suggest that Cottonmouths in East Texas may not need to aestivate during the summer and are less influenced by environmental noise. Further, they have been observed to hibernate during the cold winter months still allowing for a predictable band formation (Ford, pers. comm.).

Considering the complications with validation of skeletochronology in East Texas, it is not surprising that no differences were found between either of the sites for age-structure and body-length. Even expected differences were insignificant due to this limited sample size. All previous studies of the western ribbon snakes, for example, have concluded that males are on average smaller than females. Therefore we expected the mean age for males from both populations to be significantly smaller than for females.

From these findings, it is not possible to state conclusively that flooding is or is not influencing the age-structure or other life-history traits of western ribbon snakes in East Texas. Further studies with larger populations sizes need to be performed to allow a more accurate picture to emerge. To accomplish this task, more sites might be selected in addition to Camp Tyler and the OSBWMA. In order to validate skeletochronology and test the age-structure effects of stochasticity, I suggest the use of a more hearty snake species that maintains summer surface activity, is tolerant of extreme conditions, and still shows variation in life-history traits such as the western cottonmouth (*Agkistrodon piscivorus*).

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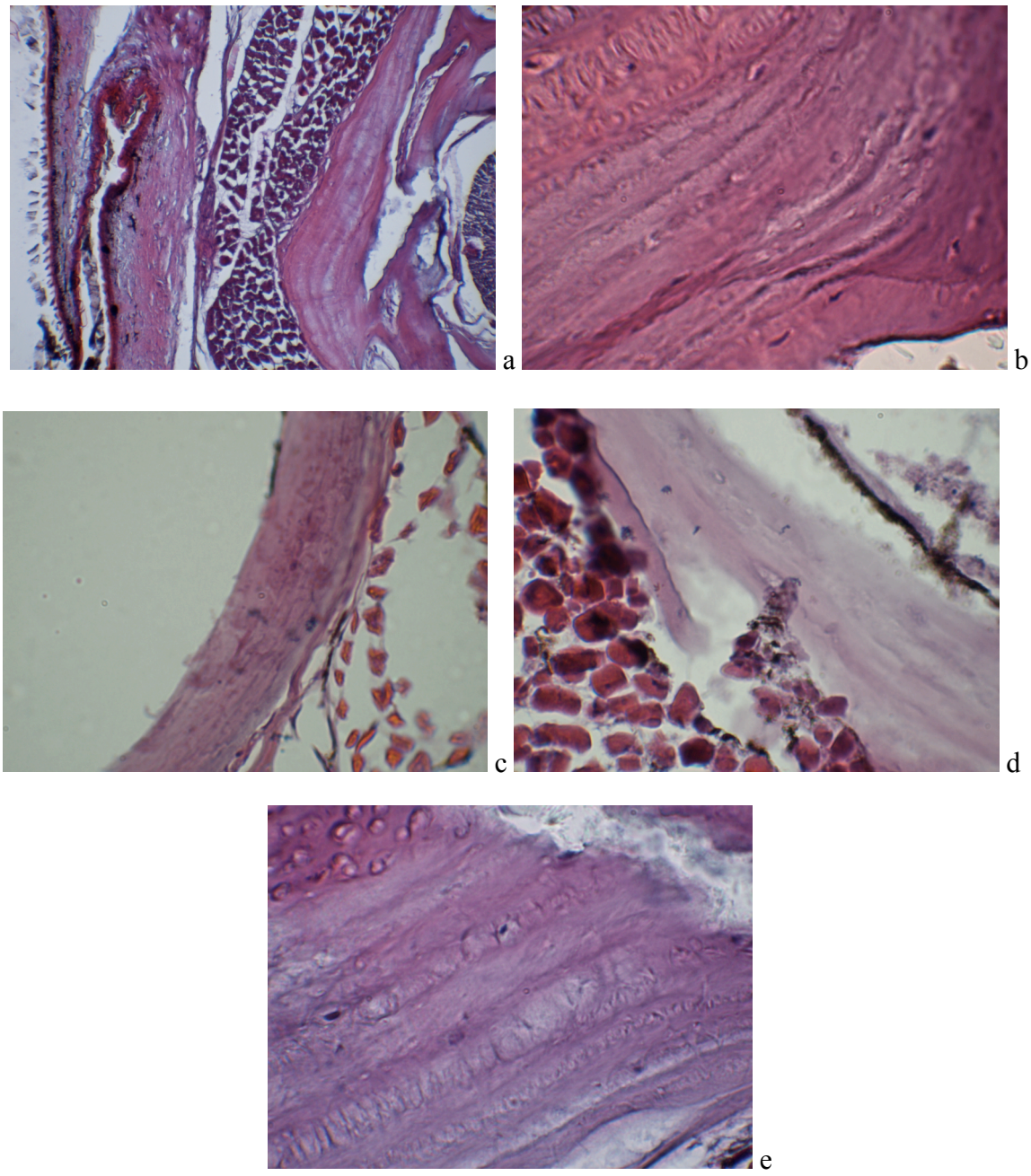
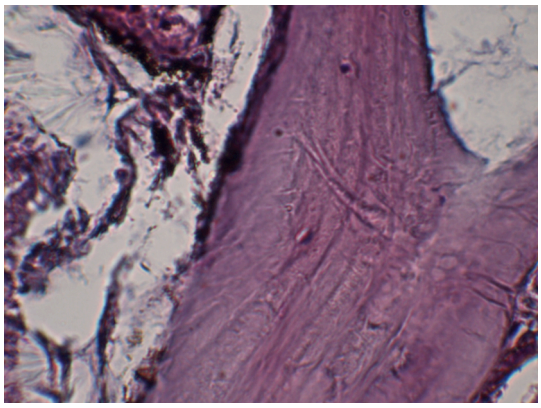


Figures 2-1 a-e. These images show distinct skeletochronology banding pattern in all species sampled in this study. Banding of a western cottonmouth adult (*Agkistrodon piscivorus*) collected from the OSBWMA (Figure 1a). Banding of a broad-banded watersnake (*Nerodia fasciata*) juvenile collected from the OSBWMA (Figure 1b). Banding of a yellow-bellied watersnake (*Nerodia erythrogaster*) juvenile with a prominent yolk scar from the OSBWMA (Figure 1c). Banding of a Texas brown snake (*Storeria dekayi*) juvenile with a prominent yolk scar from the OSBWMA (Figure 1d). Banding of an adult western ribbon snake (*Thamnophis proximus*) collected from Polk County, Iowa.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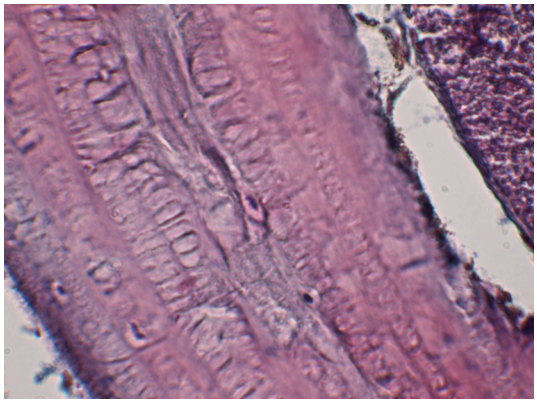
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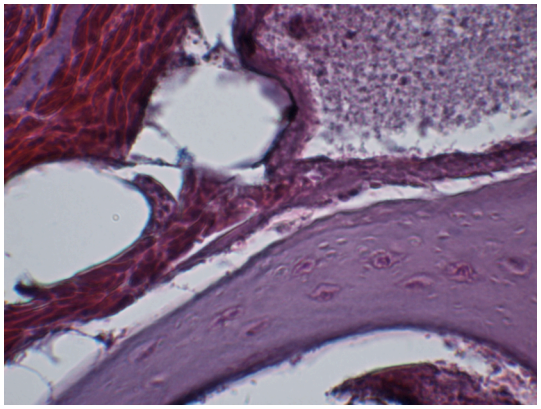
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Figures 2-2 a-c. These images are banding of western ribbon snakes caught within the study sites and born in lab for comparison. The banding of an adult female ribbon snake captured at the OSBWMA showing 6 distinct LAG's (Figure 2-2a). The banding of an adult male ribbon snake captured at Camp Tyler showing 7 LAG's (Figure 2-2b). A neonate ribbon snake born in captivity. This individual shows no banding and distinct osteoclasts found in newly formed bone (Figure 2-2c).

Table 2-1. A summary of the mean age and body length of all snakes caught at the Old Sabine Bottoms Wildlife Management Area and Camp Tyler during this experiment.

	N	Mean Age	SD	Mean SVL	SD
Sabine Female	25	4.4	3.1	50.1	15.9
Sabine Male	26	4	2.4	40.3	10.5
Tyler Female	3	2	1.0	40.8	14.6
Tyler Male	8	3.3	2.0	42.6	8.9

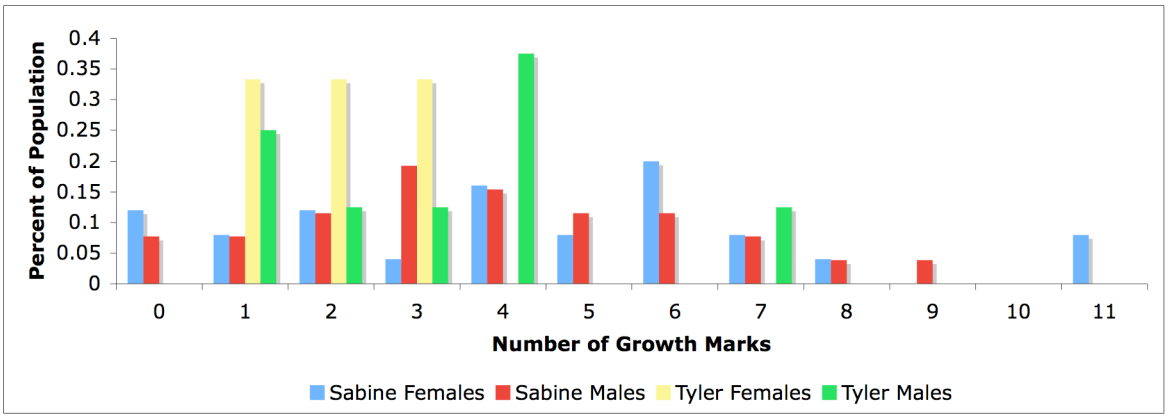


Figure 2-3. The percent of each population (Sabine Females, Sabine Males, Tyler Females, Tyler Males) in each age-class

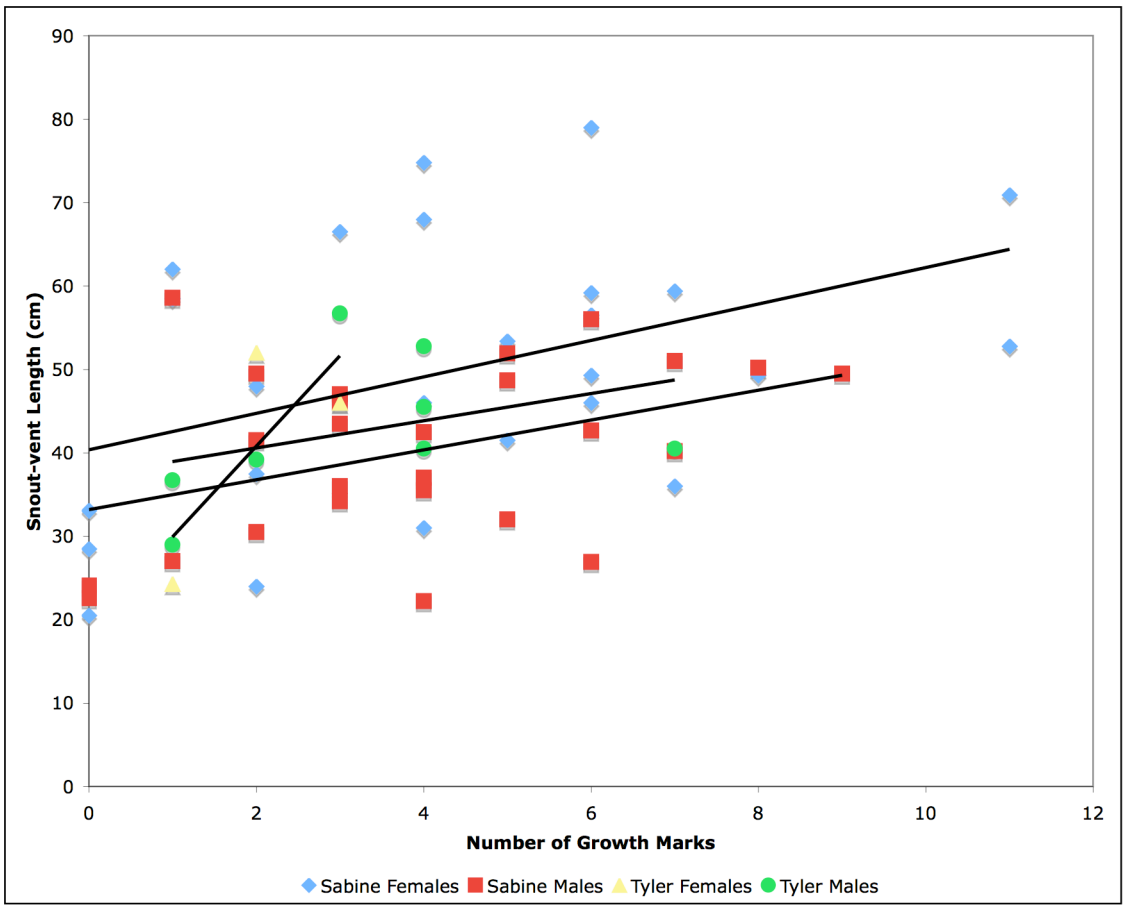
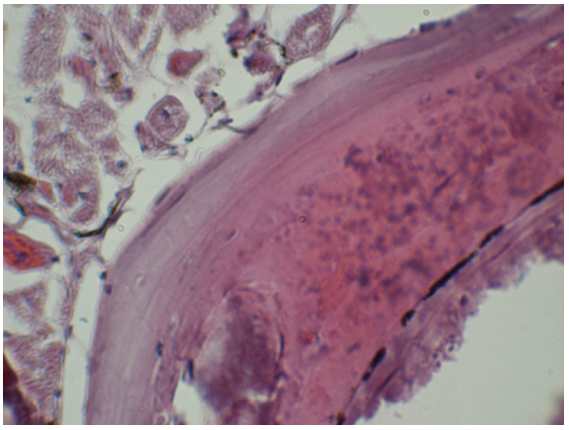


Figure 2-4. Linear regressions of the number of growth marks assessed from the bone against their snout-vent length. Their respective regression coefficients are as follows: Sabine Females: R^2= 0.180, Sabine Males: R^2= 0.163, Camp Tyler Females: R^2= 0.553, Camp Tyler Males: R^2= 0.133.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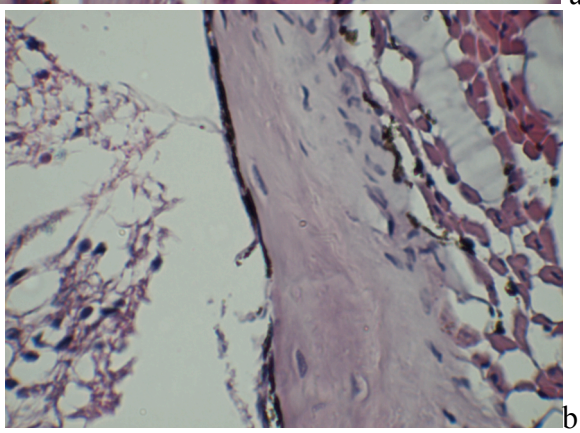
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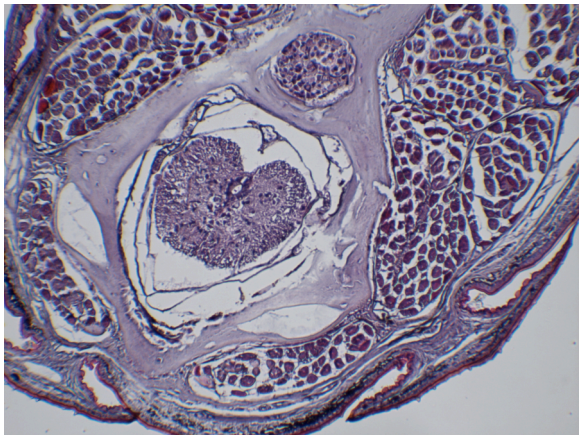
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Figure 2-5 a-c. These images represent neonates that have completed their first hibernation and should have one LAG or annulus. Two of them had prominent yolk scars to confirm the age. The third was the correct size and caught in the same month but a prominent yolk scar was not recorded (though it may have been present). A neonate ribbon snake that has completed one hibernation and was captured in April at the OSBWMA. It shows a distinct annulus and growth zone, as predicted and had a distinct yolk scar (Figure 2-5a). A neonate ribbon snake that has completed one hibernation and was captured in April at Camp Tyler. It shows a less distinct annulus/growth zone and had a distinct yolk scar (Figure 2-5b). A neonate ribbon snake that has completed one hibernation and was captured in April at the OSBWMA. No annulus was documented on this individual and a yolk scar was not recorded (Figure 2-5c).

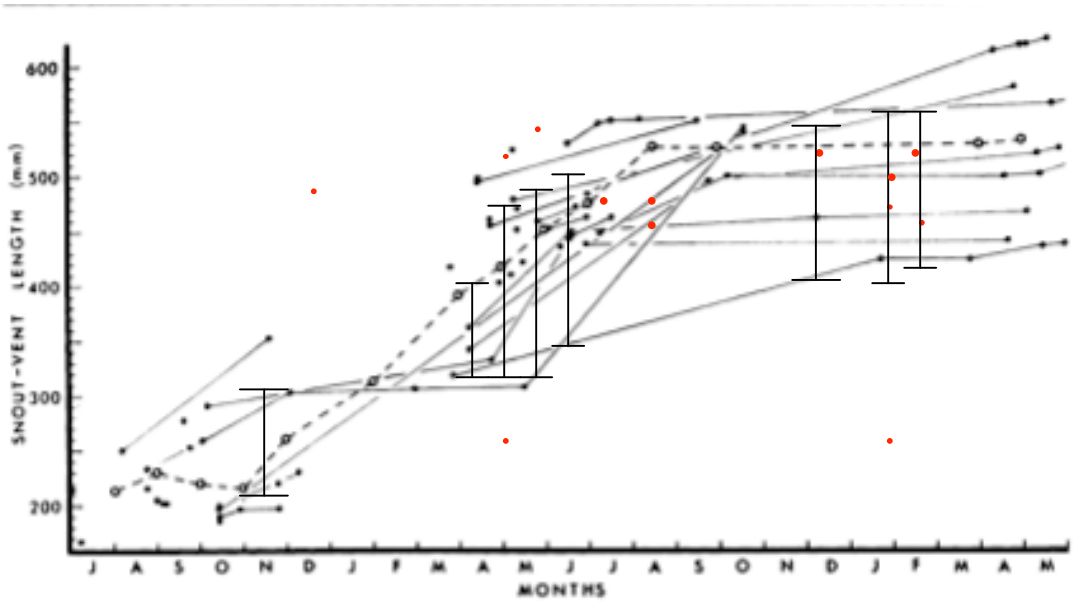
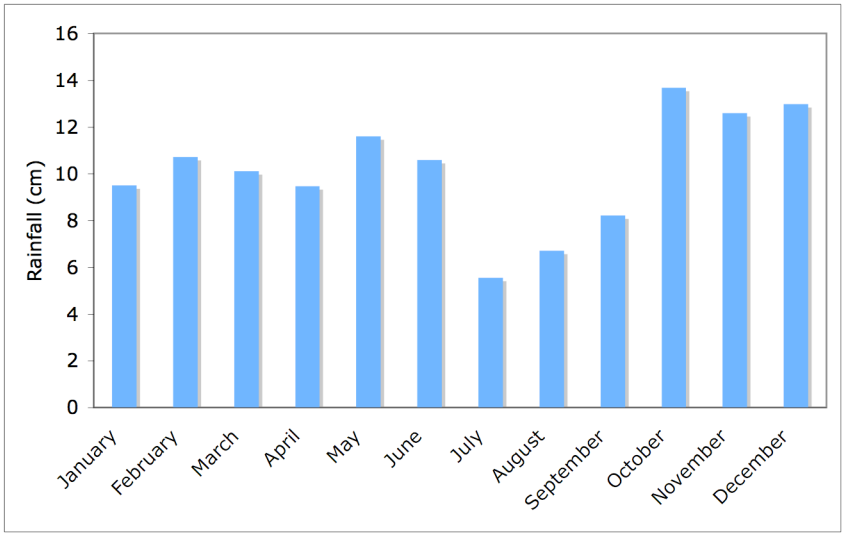


Figure 2-6. Clark's growth curve (1974) using mark-recaptures of neonate female ribbon snakes captured in Brazos County, Texas, overlaid with my age estimations of snakes with similar sizes and capture dates to compare known growth data. Gray dots and lines represent Clark's original data, orange dots represent my data. Bars representing the variation at each month in Clark's data were added later, since Clark did not provide a measure of variation.

Table 2-2. Captures by month from five separate collections across the state of Texas. Ford (unpub. data), Hampton (unpub. data), and my data were collected at the OSBWMA. Clark (1974) collected from a population in Brazos County, TX. The museum represents the Texas A&M University Natural History Museum collection which has specimens collected from across the state.

Snake Captures by Month												
Collector:	**J**	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**
Ford	0	4	9	24	27	33	13	18	5	1	2	2
Chamberlain	0	1	6	18	22	11	0	0	4	5	0	0
Hampton	0	1	3	54	32	4	1	3	3	2	4	1
Clark	1	0	7	15	16	10	15	4	6	3	2	2
Museum	10	8	75	162	100	87	42	69	25	19	20	1



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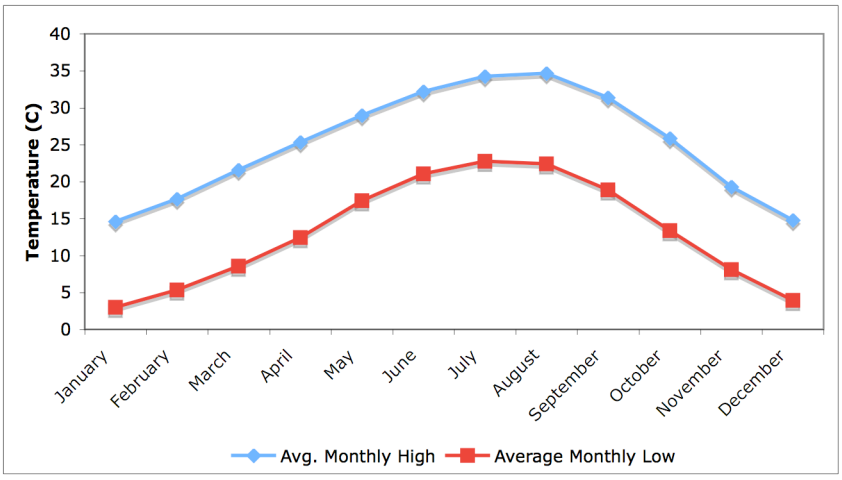

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Figures 2-7 a-b. These graphs are mean climate conditions in East Texas. The top figure represents the mean monthly rainfall for East Texas (estimated at Mineola, Wood County, Texas)(Figure 2-7a). The bottom figure shows mean daily highs and lows for each month for East Texas (estimated at Tyler, Smith County, Texas)(Figure 2-7b). All data were gathered from the United State Weather Service website from 1976-2011.

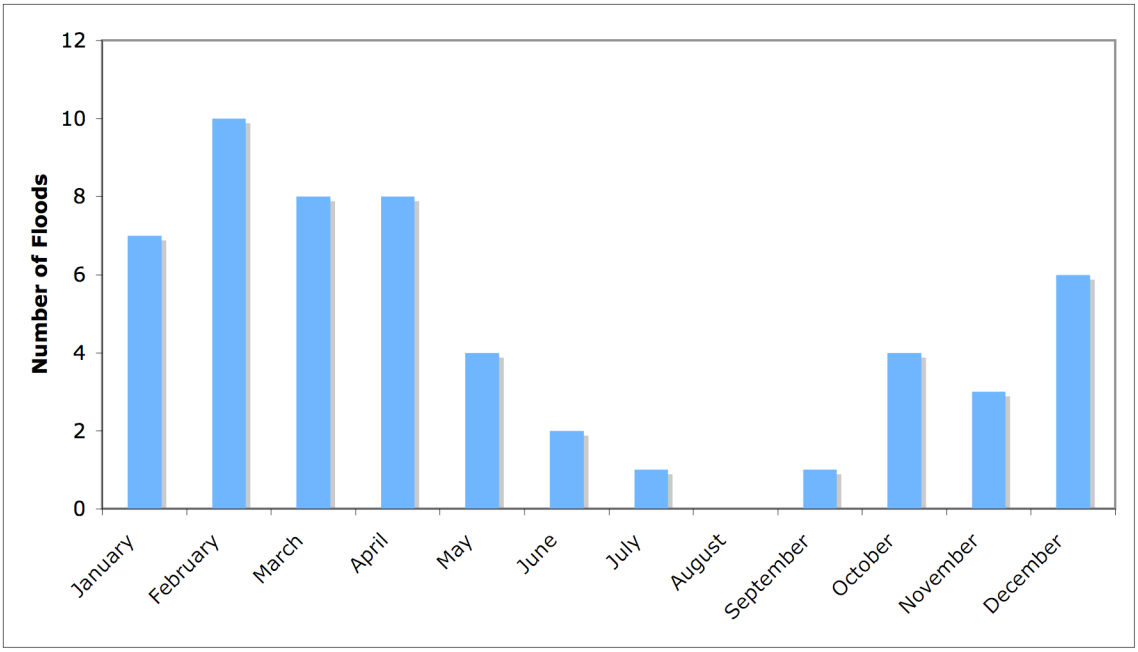

Figure 2-8. Total number of floods were calculated for each month at the USGS river gauge in Mineola, Texas above the OSBWMA from 1976-2010. A flood was defined as any measured stage above the USGS defined flood stage. Data were collected from the United States Geological Survey website between the years of 1976-2011.

Chapter Three

Introduction

Environmental stochasticity occurs in many forms including floods, droughts, storms, fires, etc. and to different degrees. These events can cause drastic changes to the surrounding landscape and resources available to organisms living there. Habitats available for reproduction, foraging, and thermoregulation all might change. Similarly, available prey species and abundances may shift, causing changes in food availability for predators. This variation in resources can play a major role in shifting organism's strategy for survival and reproduction (Benton et al., 1995; Marion et al., 2000; Saether, 1997).

Alterations to food availability in particular has been linked with shifts in reproductive traits (Winne et al., 2006). Increases in prey availability allow predators to correspondingly increase their caloric intake. This additional energy can theoretically be shuttled towards reproduction through various pathways. Thus, some organisms like snakes can vary their reproductive traits, taking advantage of good years for food availability and limiting their reproduction during bad ones (Madsen & Shine, 1999a).

One ecosystem that is particularly susceptible to stochastic food availability is floodplains. These habitats are periodically inundated with water that alters habitat, saturates the soil forming extensive pools that are ideal for breeding of some species, and drives other inhabitants to seek refuge from the cascade of water. Often, after a flood event there can be a burst in the populations of certain animals that depend on floods to complete their life-cycle, such as insects, fish, and amphibians. Many predators take

advantage of this prey influx and will increase their foraging and caloric intake. Increased food intake, as mentioned before, can result in shifts in reproductive effort of these predators.

This relationship between floods, increased prey, increased predation, and higher reproduction has been documented in snakes previously, particularly by the work of Madsen and Shine using water pythons (*Liasis fuscus*) (Madsen & Shine, 1999a: Madsen & Shine, 1999b; Madsen et al., 2006; Shine & Madsen, 1997). Using more than a decade of data, these researchers clearly showed that pulses in the python's rat prey allowed changes in reproduction in the water pythons due to increased predation. Pulses in rat populations were strongly correlated with the presence or absence of floods at their study site (Madsen & Shine, 1999c).

This study attempts to show similar correlations between floods, prey, predation, and reproduction of a different species of snake, the western ribbon snake (*Thamnophis proximus*). These snakes occur in North America and Central America and feed heavily on fish and amphibians, rather than rats such that their pythons consume (Ernst & Ernst, 2003; Rossman et al., 1996). Here we utilized a population of these snakes occurring in the floodplain of the Sabine River in the Old Sabine Bottoms Wildlife Management Area (OSBWMA), Smith County, Texas. Hampton and Ford have previously shown that flood suppression in this site results in a decrease in prey abundances (fish, frogs, and lizards) of these snakes (Hampton & Ford, 2007). I propose therefore, that in years with fewer floods, the western ribbon snake's food is limited, resulting in a decrease in reproduction, measured by mean offspring SVL (snout-vent length), mean offspring mass, clutch size, and clutch mass.

Methods

Between the years of 2002-2005 and 2010, fifty gravid female western ribbon snakes were collected from the OSBWMA, Smith County, Texas. These individuals were brought back to the lab where they were allowed to give birth. Females were offered tadpoles and/or adult frogs of several species once a week during the course of their gestation. Reproductive traits were measured on the gravid females and their offspring including: clutch size, clutch mass, average offspring SVL, average offspring weight, and SVL of the female. All females and their offspring were released back to the wild to the their respective sites of capture.

The OSBWMA is a 2,087-hectare reserve run by the Texas Parks and Wildlife Department and is located within the floodplain of the Sabine River. It is predominantly bottomland hardwood forests supporting a large population of *Thamnophis proximus*. Flooding typically occurs in winter months, and completely inundates the bottomland forest. As waters recede, pools form, trapping large amounts of potential prey items for ribbon snakes, particularly amphibians and fish. Most of these ponds will dry up by the end of the summer (Ford, pers. comm., pers. obs.). Food availability therefore can fluctuate in response to how recently a flood event has occurred and the length of time pools with food will remain if flooding did occur.

Flooding was estimated using the USGS (United States Geological Survey) river gauge station on the Sabine River near Mineola, TX, the closest station above the OSBWMA. Floods were defined as any stage measured above the USGS defined flood stage for that station. The total number of peaks above flood stage was counted for a year before the average date of birth for the gravid females. A year before the birth was used

because it assumes all the floods and feeding opportunities that happened after the previous reproductive event. The average date of birth was assumed as the same across all years. This date was estimated using data of ribbon snakes from Lancaster and Ford, who also sampled from the OSBWMA (Lancaster & Ford, 2003).

Multivariate Analysis of Variance (MANOVA) was used to assess differences between years for four reproductive traits: clutch size, clutch mass, mean offspring SVL, and mean offspring weight. A Tukey-Kramer post-hoc test was used to analyze which years were specifically different. An Analysis of Variance (ANOVA) was performed to see if gravid females' SVL's were different between years. Multiple linear regressions were utilized to assess any trends between number of floods and any reproductive characteristic. These were also used to estimate correlations between the gravid female SVL and any reproductive characteristic. Regression coefficients were compared to evaluate the degree of correlation between these variables.

Results

A summary, including means, standard error, minima, and maxima can be seen in Table1. The data were pooled from all years of the study.

Reproductive characteristics of *Thamnophis proximus* did in fact vary between years as would be expected given my hypothesis (Wilk's lambda= 0.271, $F_{16, 126} = 4.19$, $p<0.001$). Only mean offspring SVL ($F_{4, 44} = 5.940$, p= 0.002) and mean offspring weight ($F_{4, 44}= 0.314$, p= 0.27) significantly varied between years. Tukey-Kramer post-hoc tests indicated that mean offspring SVL in 2010 was different than 2002, 2004, and 2005 (critical value= 3.214, alpha= 0.05). However, the Tukey-Kramer test failed to detect any

yearly difference for mean offspring weight, although other less conservative tests did detect a difference (critical value= 4.022, alpha= 0.05).

There was a difference in the SVL of gravid females between years ($F_{4, 44} = 3.44$, p= 0.016). Tukey-Kramer post-hoc tests showed a difference between 2002 and 2010 for female SVL (critical value= 4.022, alpha= 0.05). Furthermore, female SVL was positively correlated with both clutch size ($R^2= 0.36$) and clutch mass ($R^2= 0.45$) as is expected from previous studies but not with mean offspring SVL or mass (Figures 1-4).

While there is evidence of a yearly difference in reproductive traits, this difference cannot be accounted for by the number of floods up to a year before the average date of parturition for gravid females. There was very little correlation between number of floods and all four reproductive characteristics measured (Offspring SVL multiple $R^2= 0.025$; Offspring Mass multiple $R^2= 0.059$; Clutch Size multiple $R^2= 0.001$; Clutch Mass multiple $R^2= 0.006$) (Figures 5-8).

Discussion

Floods and by extension food availability, do not seem to play a significant ecological role in the ear-to-year variation of reproductive life-history traits in the western ribbon snake (*Thamnophis proximus*). While there was in fact yearly variation in some of these traits, the number of floods was not able to explain a large portion of this difference. It appears that SVL of the female has a more important role in varying reproductive traits year to year than does food availability.

Yearly differences were detected in only two of the four reproductive characteristics measured, mean offspring SVL and mean offspring mass. It is not surprising, however, that these two traits varied together, as it has been well established

that offspring mass and offspring SVL are correlated in many ectothermic vertebrates, although this relationship may not always be predictable (Halliday & Verrell, 1988). The factors driving variation in the size of the offspring, while having no affect on clutch size or overall clutch mass is no well understood. These inconsistencies may be due to variation in food availability between years.

In an attempt to understand intra-annual variation in reproduction, the influence of stochastic food availability was examined. With increased consumption of prey, it is assumed that females should be able to shift to larger clutches with larger offspring due to increased energy intake. To estimate food availability, the number of floods during a reproductive year was used. In floodplain habitats, floods often determine the life-cycle of prey items found there, thus changing the available prey for larger predators. Since the number of floods can vary each year, it was assumed that prey availability would consequently also vary each year. Changes in flood regime of the OSBWMA have already been shown to change prey availability for the western ribbon snake (Hampton & Ford, 2007).

However, there is little evidence that the number of floods influences mean offspring SVL, mean offspring mass, clutch size, and clutch mass in predictable linear fashion. This indicates that other more important factors may be at play causing the year-to-year variation in reproduction that was detected. These factors may include the duration of flood periods, the timing of flood periods, or the size of the females breeding that year. Our results stand in contrast to the studies of Shine and Madsen that found that variation in reproduction was closely correlated with variation in food availability due to rainfall (Madsen & Shine, 1999a; Madsen & Shine, 1999c; Madsen et al., 2006).

The role that SVL of the female plays on variation in reproduction may be substantial, which has been demonstrated in other snake species (Ford et al., 2004; Madsen & Shine, 1996a). We detected a strong difference in the mean SVL of gravid females between years. Additionally, female SVL was strongly correlated with both clutch size and clutch mass. Clearly some factor or factors influence the size of females breeding each year, although what those factors are is unclear. Some of this variation may be explained by bias introduced from uneven capture of different sized females between years.

Further studies are required to truly understand this complex relationship between stochastic food availability and reproduction. Food availability may not be simply a function of the number of floods occurring in a year but may be influenced by duration, extent, and timing of these floods. A particular focus should be paid to long-term studies as stochasticity in food availability and changes in flood cycles are doubtless long-term phenomena, as many climatic patterns can take years to decades to shift. Additionally, these factors need to be studied within the context of other phenomena that may influence reproduction such as competition, prey type, and climate.

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Table 3-1. A summary of the means all reproductive traits between the five years measured, including the number of floods that occurred that year

Year	*n*	Female SVL (cm)	Off. SVL (cm)	Off. Mass (g)	Clutch Size	Clutch Mass (g)	# of Floods
2002	16	55.8	17	1.91	11	20.8	6
2003	11	62	17.8	2.06	11	22.2	5
2004	8	59.4	16.9	1.64	12	19.6	2
2005	9	62.3	16.7	1.68	12	20.3	2
2010	5	65.3	19	1.69	15	25.4	6
Total	49	59.9	17.3	1.83	12	21	4

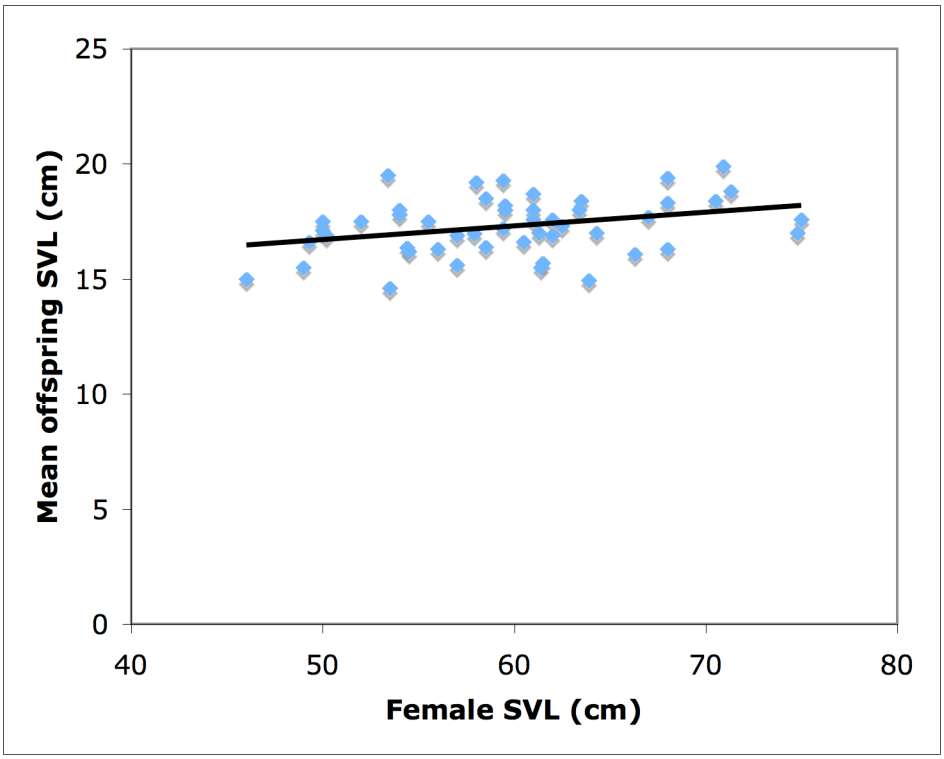

Figure 3-1. A linear regression of female ribbon snake SVL from all years to mean offspring SVL (R^2= 0.107)

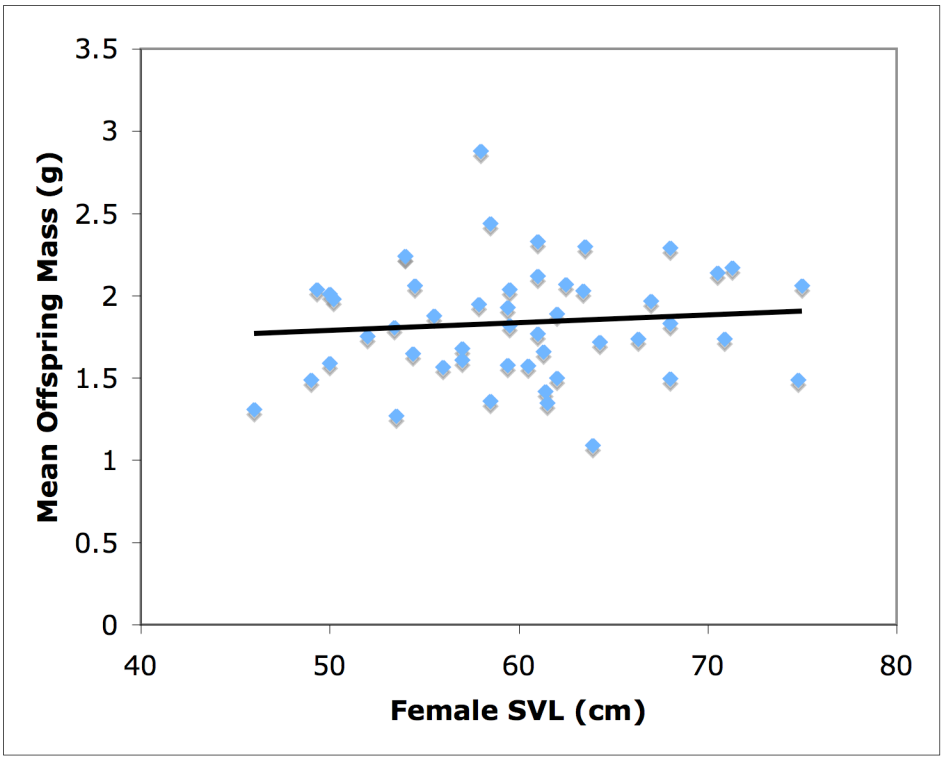

Figure 3-2. A linear regression of female ribbon snake SVL from all years to their mean offspring mass (R^2= 0.009)

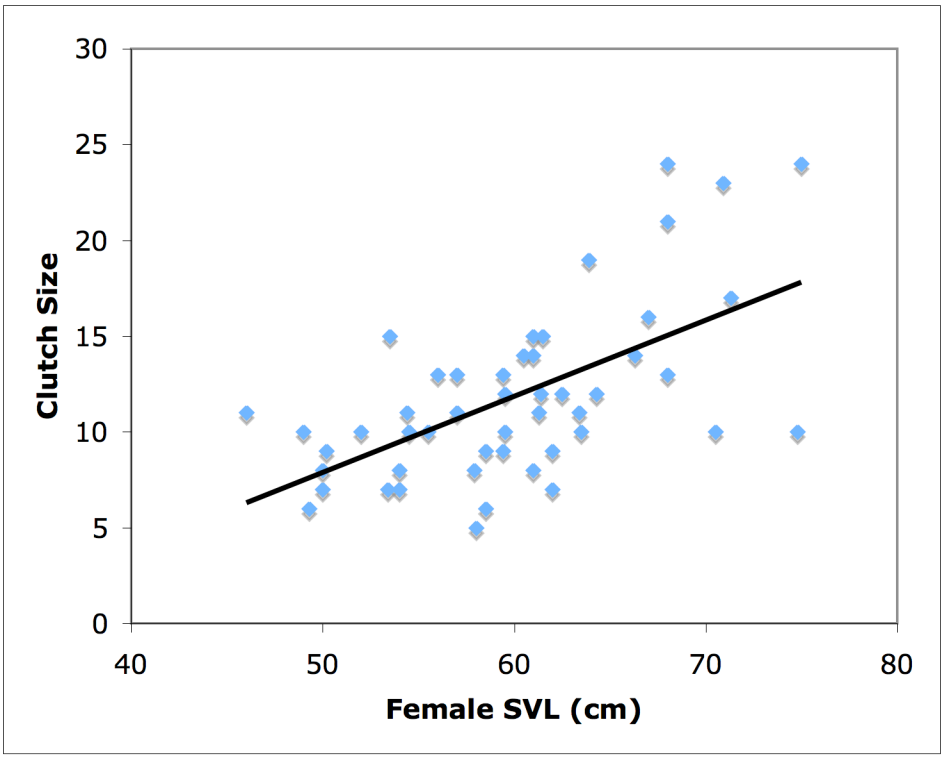

Figure 3-3. A linear regression of female ribbon snake SVL from all years to their clutch size (R^2= 0.360). There was significant correlation between these variables.

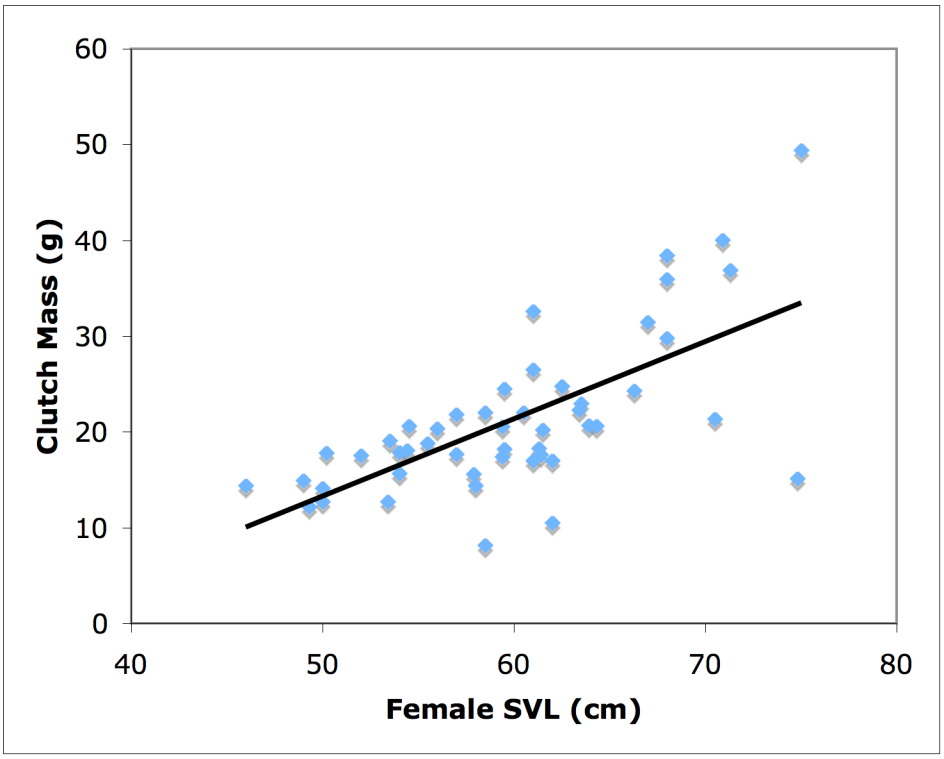

Figure 3-4. A linear regression of female ribbon snake SVL from all years to their clutch mass (R^2= 0.450). There was significant correlation between these variables.

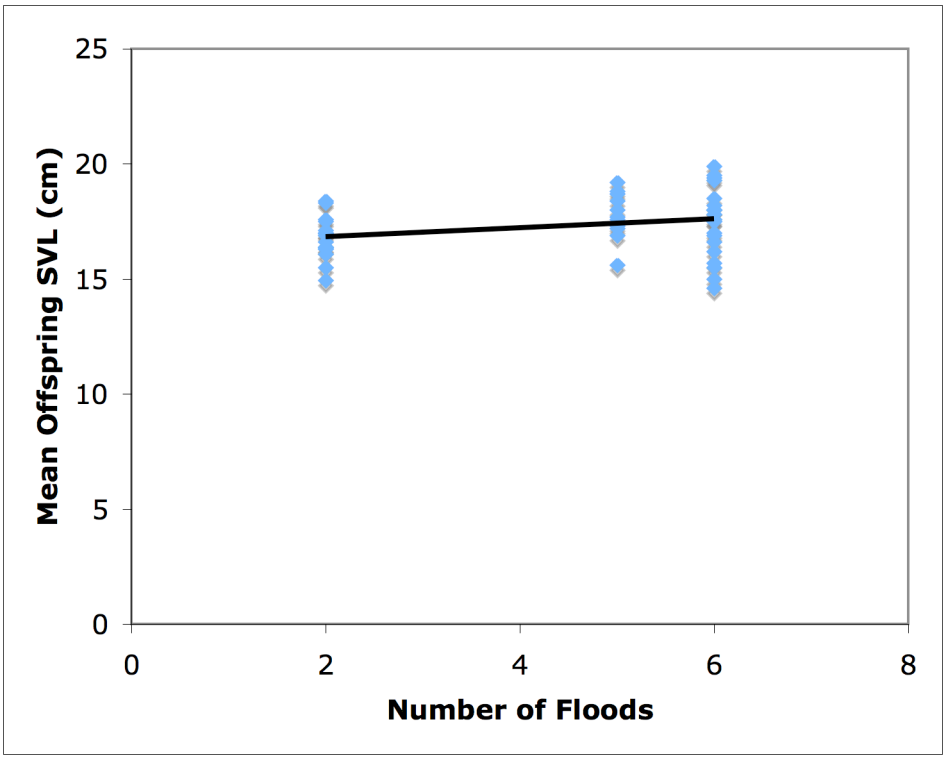

Figure 3-5. A linear regression of the number of floods a year before the mean date of birth for all females across all years to their mean offspring SVL (R^2= 0.025)

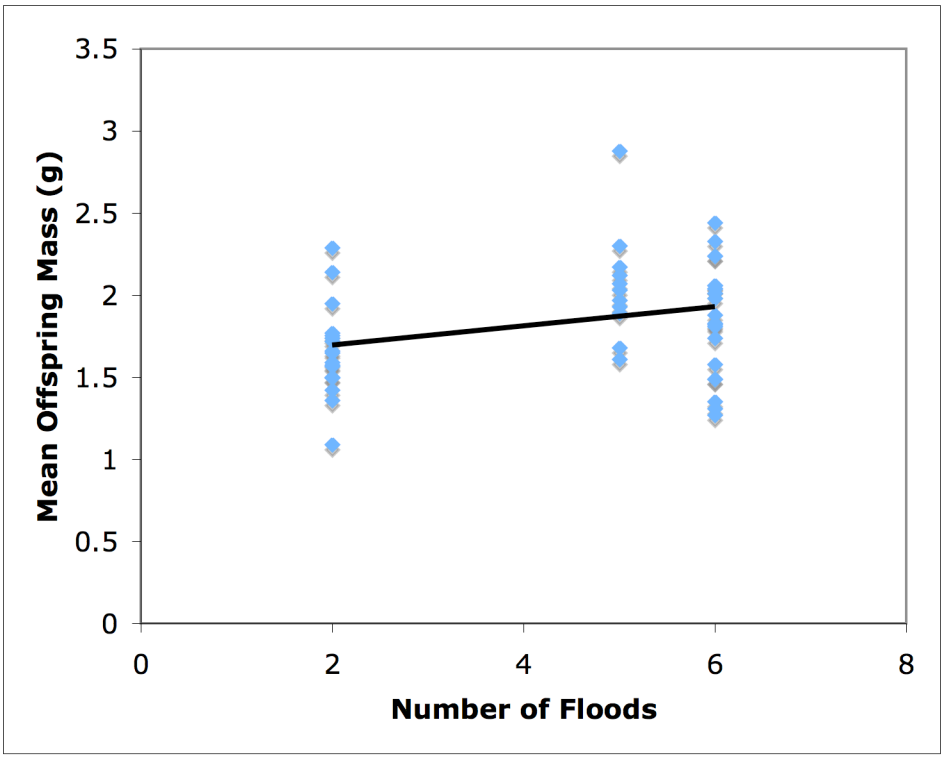

Figure 3-6. A linear regression of the number of floods a year before the mean date of birth for all females across all years to their mean offspring mass (R^2= 0.059)

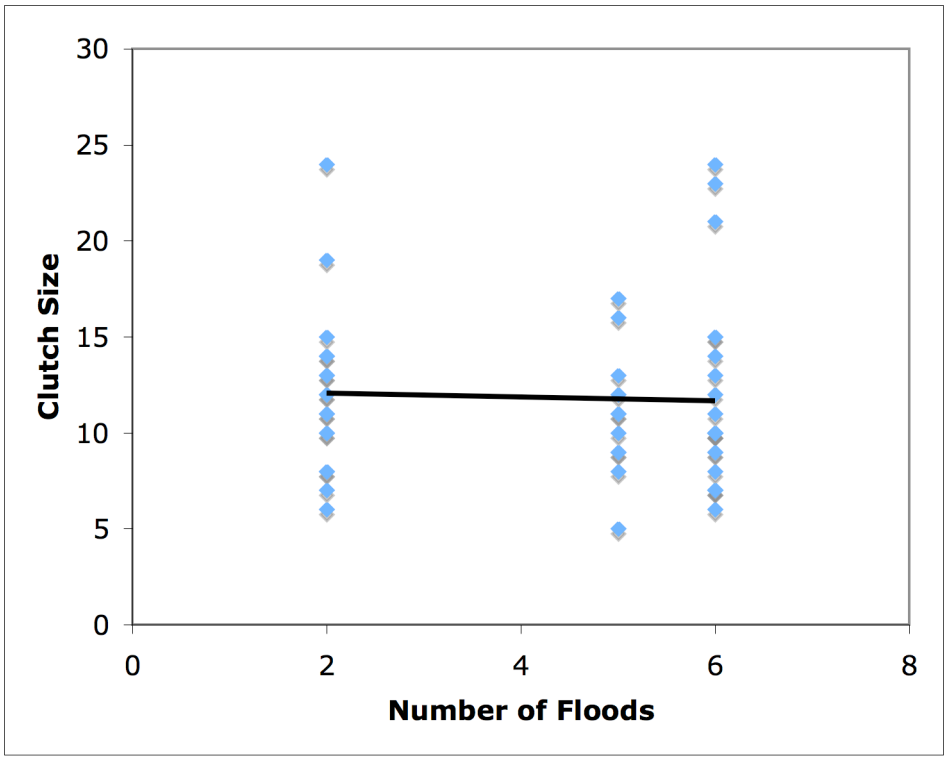

Figure 3-7. A linear regression of the number of floods a year before the mean date of birth for all females across all years to their clutch size (R^2= 0.001)

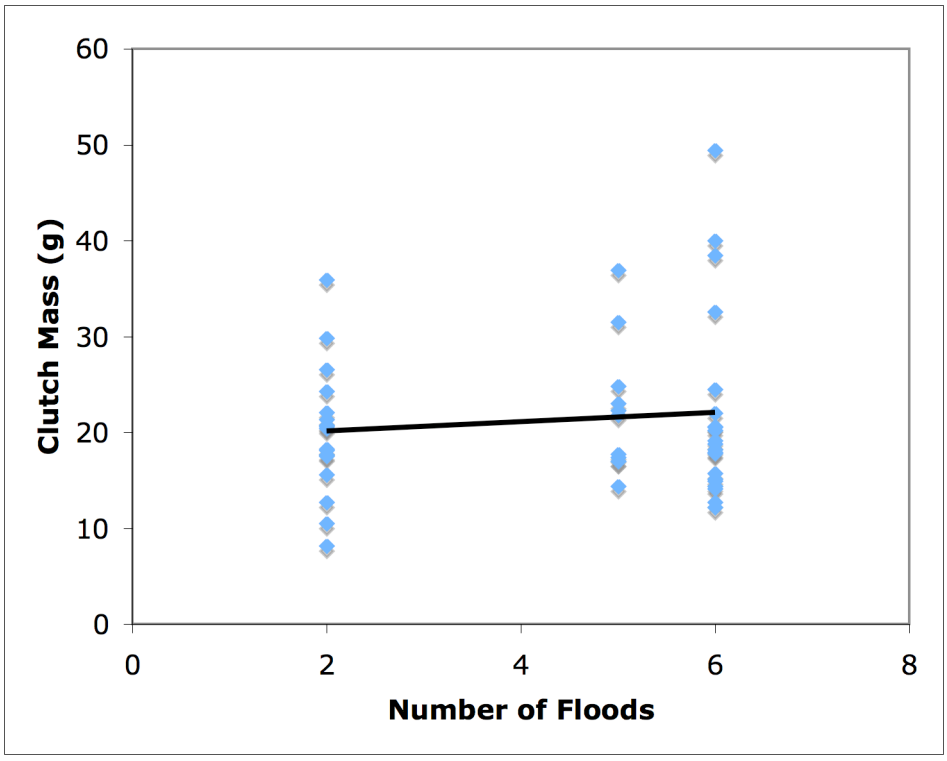

Figure 3-8. A linear regression of the number of floods a year before the mean date of birth for all females across all years to their clutch mass (R^2= 0.006)